Review Article

A Review of Antifungal Activity of Combined Plant Extracts or Plant Exudates from Medicinal Plants either together or with Known Antifungal Agents

Abstract

Medicinal plants provide humanity with important phytochemical compounds and extracts which are widely used in treatment of many diseases. Fungal infections are one of these diseases which are widely distributed especially in developing countries, in which medicinal plants are extensively used in developing countries. There are few antifungal agents, most of them are expensive and have many adverse effects, also there is high incidence of drug resistance among some available antifungal agents, hence for these reasons many people, especially in developing countries, use medicinal plants (either alone, combined together or combined with available antifungal agents) in treatment of many fungal infections. This rise a new and important issue about plant(s) – plant(s) and plant(s) - drug interactions.

The aim of this review is to try to fill the gap of understanding the interactions of plant(s) - plant(s) and plant(s) – drug(s) combinations by providing an overview of some evidence-based researches done in this field. We collected a lot of studies which studied the interactions between plant(s) (including extracts, isolated active constituents, essential oils, plants latexes and other phytochemicals) used either together or with conventional antifungal agents. This will not only bring about better understanding of both phytochemicals and antifungal activity, but also may help in searching and developing new drugs which should be safe and effective in treatment of fungal infections.

Key words: Antifungal activity, plant-plant combination, plant-drug combination, synergism.

Introduction:

The relationship between man and plants has always been a very close one throughout the development of human culture, and no doubt, the herbalist is probably one of the first professionals in the evolution of human cultures. Today, the plant kingdoms still remains a virtually untapped reservoir of new compounds, some provide novel structures from which synthetic chemists may derive even more interesting compounds (El Ghazali *et. al.*, 2003).

Over the last few decades, there has been a considerable interest worldwide in traditional and complementary medicine, specifically in herbal medicines. The World Health Organization (WHO) also described the main role of herbal medicines in preventive, promotive, and curative healthcare system, especially in under- developed countries (WHO, 2013).

A survey made in 2007 demonstrated that 15% of the patients receiving conventional pharmacotherapy also take herbal products. Among these, potential herb-drug interactions were observed in 40% of the patients, but it is often difficult to establish

the causative agent of a herb-drug interaction, especially if it occurs in patients receiving multiple drug therapies. This is a substantial public health problem since many patients who are known to be using one or more prescription drugs are also taking supplement preparations, and unfortunately only about one third of these patients were reported to tell their physician about their use of these products (Gül Dülger, 2012).

There is a general belief by the public that herbal medicines are safe because they are natural. However, this is a hazardous over simplification. Many different interactions and side effects to herbs have been reported and recently reviewed (Kennedy and Seely, 2010).

The combinations of two or more phytochemicals bring about changes in the ultimate biological effects and/or the bioavailability of each component. A number of mixtures of pure bioactive compounds or phytochemical-containing plant extracts provide synergy with regard to antioxidant status, anti-inflammation anti-cancer and chemoprevention of several oxidative stress and metabolic disorders *in vitro* (Phan *et. al.*, 2018). In some cases, however, the combination of phytochemicals may lower the biological effects if they are combined in inappropriate ratios (Iwuchukwu *et. al.*, 2011). The interactions of phytochemicals may enhance or reduce the bioavailability of a given compound, depending on the facilitation/competition for cellular uptake and transportation taking place between them (Reboul *et. al.*, 2007). Bioactive compounds mixtures may produce a biological effect higher or lower than the summative effects of each single component. The effects of phytochemical interactions can be classified as potentiation, addition, synergy, or antagonism. There is confusion in literature about the differences between potentiation and synergy (Efferth and Koch, 2011). If the phytochemical mixture containing two compounds, in which one is active and the other is inactive, produces a greater effect than that of its single active component, the effect is defined as potentiation: the presence of the inactive compound enhances the potency of the active one (Efferth and Koch, 2011). If each component of the mixture is active, their mixture can produce an additive, synergistic or antagonistic effect (Chou, 2006). Synergy comes from the Greek word “synergos”, which means “working together.” More precisely, synergism or synergy according to the McGraw–Hill Concise Dictionary of Modern Medicine is indicated as “the cooperative interaction between two or more components of a system, such that the combined effect is greater than the sum of each part” (Pezzani *et. al.*, 2019). Synergy is broadly defined as the interaction or cooperation of two or more substances, organizations or other agents to produce a combined effect greater than the sum of their separate portions (Mirghani *et. al.*, 2018). Because the herb extracts consist of complex mixtures of major compounds, concomitant agents and other substances, the complex multi-component nature of medicinal herbs may serve as a valuable resource for network-based multi-target drug discovery due to its potential treatment effects by synergy. For instance, polyphenols and terpenoids are two groups of constituents which are contained in many plant extracts; the former possess a strong binding ability to different molecular structures like proteins or glycoproteins, while the latter have great affinities for cell membranes and therefore, a high potential to permeate through cell walls of the body or bacteria (Wagner and Ulrich-Merzenich, 2009). In this regard, synergistic expects may be observed in the interaction between herbal products and conventional drugs or biochemical compounds (Pezzani *et. al.*, 2019). In fact, a combination of two or more active chemicals can produce an additive

(combined effect is equal to the sum potency of individual components of the mixture). Antagonistic is when combined effect is less than the sum potency of individual components of the mixture (Zhang *et. al.*,2019).

Fungi have a major influence on the health and livelihood of mankind. Diseases caused by fungi are termed mycoses, and range from very common mild chronic infections, fungal infections today are among the most difficult diseases to manage in humans. (Köhler *et. al.*, 2017). Among the estimated 1.5–5.0 million fungal species on planet Earth, only several hundred cause disease in humans, and very few are able to affect healthy people (O'Brien *et al.*, 2005). Important progress has been achieved in an understanding of fungal pathogenicity including the mechanisms of adherence to host tissues, penetration of tissues, multiplication within the host and the interaction of fungal cells with host effector cells. In addition to the increase in infections by opportunistic and pathogenic fungi in compromised patients, caused by *Candida* spp., *Aspergillus* spp., *Cryptococcus neoformans*, *Histoplasma capsulation* and *Coccidioides immitis*, many fungi that occur as saprophytes in the environment and which had previously been considered to be nonpathogenic are now being encountered as causes of human infection (Malcolm D. Richardson, 1991).

Mammalian hosts may acquire a fungal infection in three ways; firstly, they may be exposed to truly pathogenic organisms that normally occur as saprophytes in the environment; secondly, individuals who are immuno-suppressed may acquire a fungal infection following exposure to weakly pathogenic organisms that occur as saprophytes in the environment, such organisms are termed opportunistic pathogens, infections such as aspergillosis and candidosis are being seen increasingly in immuno-compromised patients, particularly those with haematological malignancies or the acquired immune deficiency syndrome (AIDS); thirdly, individuals may be exposed to infective propagules of the dermatophyte fungi, organisms which are very well adapted to parasitism and quite capable of invading the healthy host, occasionally, dermatophyte fungi are found on the skin and scalp of individuals in the absence of symptoms, this is thought to represent transient colonization or a carrier state (Malcolm D. Richardson, 1991).

Fungal infections can be classified into a number of broad groups according to the initial site of infection, this brings out clearly the degree of parasitic adaptation of the different groups of fungi and the way in which the site affected is related to the route by which the fungus enters the host:
(i) The superficial mycoses : These are infections limited to the outermost layers of the skin, the nails and hair, and the mucous membranes.
(ii) The subcutaneous mycoses: these are infections involving the dermis, subcutaneous tissues and adjacent bone.
(iii) The systemic mycoses: these are infections that usually originate in the lungs, but may spread to many other organs (Malcolm D. Richardson and David W. Warnock, 2012).
In comparison with the number of antibacterial drugs available, there are far fewer antifungal compounds. Even so the number of antifungal drugs is increasing all the time. There are four major families of compounds: the polyenes, the azoles, the allylamines and the echinocandins. In addition there is a miscellaneous group of compounds, such as flucytosine and griseofulvin, which do not belong to one of the major families (Malcolm D. Richardson and David W. Warnock, 2012).

The polyenes (e.g. amphotericin B) bind to ergosterol, the principle sterol component of the fungal cell membrane resulting in a loss of cell wall integrity. The azoles (e.g. fluconazole, itraconazole, voriconazole and posaconazole) inhibit enzymes involved in ergosterol synthesis. The echinocandins inhibit glucan synthesis, glucan is a long chain polymer responsible for fungal cell wall stability, it accounts for 30–60% of the cell wall mass in *Candida*, *Aspergillus* and *Saccharomyces* species. Importantly, human cells do not contain glucan, thus accounting for the low rate of human toxicity associated with this class of agents (Van Thiel *et. al.*, 2012).
The epidemiological data suggest that the incidence and prevalence of serious mycoses continues to be a public health problem. The increased use of antifungal agents has resulted in the development of resistance to these drugs. The spread of multidrug-resistant strains of fungus and the reduced number of drugs available make it necessary to discover new classes of anti-fungal from natural products including medicinal plants. Medicinal plants have also been reported in traditional systems of medicine in the treatment of both human and animal mycoses, and are considered to be a valuable source for the discovery of new antifungal drugs (Mishra *et. al.*, 2020).

Specific Objectives:
1- To review and collect all researches done on medicinal plants combined together as anti fungal (plant(s)- plant(s) combination).
2- To review and collect all researches done on medicinal plants combined with known antifungal drugs (plant(s)- drug(s) combination, isolated phytochemical compound- drug(s) combination, essential oil- drug(s) combination and plant latex-drug(s) combination.

Methodology:
The data collection on the topic was conducted comprehensively by using international reliable databases for medical searching; i.e. PubMed (the National Library of Medicine), MEDLINE, Science Direct, Scopus, Google Scholar, Research gate, Wiley online library and other reliable databases for medical journals articles were used. Searches were not limited to publishing time.
The databases were thoroughly searched for studies that met the inclusion criteria. Search results were assessed for relevance according to the title, abstract and sometimes full text review. All abstracts were reviewed in relation to the inclusion/ exclusion criteria. Unless the abstract clearly described one or more exclusion criteria, the full article was then reviewed to check if it still met the inclusion criteria.
Any study was eligible for inclusion if it practically examined the effect of the plant(s)- plant(s) or plant(s)– drug(s) combination in term of either synergism, addition or antagonism. For all combinations (except some combinations of essential oils and phytochemicals with synthetic drugs) only studies that clearly mentioned the plants parts, extraction solvent and comprehensive results were considered as relevant.
Finally, the collected articles were reviewed one by one and the relevant information was extracted, analyzed, summarized and presented with their references.

Results:
Plant(s)- plant(s) combination:
The leaves and stems (aerial parts) of *Zuccagnia punctata* (Fabaceae), *Tetraglochin andina* (Rosaceae), *Larrea cuneifolia*, *L. nitida* and *L. divaricate* (Zygophyllaceae)

were separately macerated in ethanol. The antifungal activity was individually assayed *in vitro* against six yeast strains (*Saccharomyces cerevisiae, Candida albicans, C. glabrata, C. tropicalis, C. parapsilopsis* and *C. krusei*) obtained from vaginal exudates of patients with vaginal yeast infections. The MIC values were determined for each extract, alone and in combination between them; and the FIC index was calculated. According to the results, the synergist effect was observed with the combination between *Z. punctata / L. cuneifolia* against *C. albicans, C. glabrata, and S. cerevisiae,* and between *Z. punctata / L. nitida* and *Z. punctata /L. divaricata* extracts against *C. glabrata* (FIC index=0.5). An additive effect was observed with the combination of *Z. punctata / L. cuneifolia* against *C. tropicalis and Z. punctata / L. nitida* against *C. albicans* and *C. tropicalis* (FIC index > 0.5 in both cases). Some combinations revealed an indifferent interaction, the FIC indices varying from 1.0 to 2.5. The less active mixture was *T. andina / L. nitida*, which showed indifferent effect against all the yeasts tested (FIC between 1.1 and 2.2). The results indicate that combinations between *Z. punctata* and *Larrea* species are more effective as antifungal than between *Larrea* species and between *Larrea or Z. punctata* with *T. andina*. The best combination was between *Z. punctata* and *L. cuneifolia* since it showed a synergistic or additive effect against all the tested strains, indicating that the interaction between chemical components contained in both plant species is more effective as an antifungal activity (Moreno *et. al*., 2020).

The effect of combined aqueous, ethanolic and hydro-ethanolic extracts of different parts (stem and barks, twigs and leaves) of *Annona senegalensis* (Annonaceae) was assessed against pathogenic yeasts. The MIC of extracts on the yeasts were ranging from 0.156 to > 5 mg/ ml. Overall, *Candida krusei* was the most sensitive yeast while *Candida parapsilosis* and *Cryptococcus neoformans* were the less sensitive. Amongst the promising extracts, the aqueous extracts of the stem (StH_20), twig (TwH_20), and bark (BH_20) as well as the ethanolic extract of the leaf (LEtOH) were the most active against all the tested yeasts (*Candida albicans, Candida parapsilosis, Candida krusei* and *Cryptococcus neoformans*) with MIC values comprised between 0.312 mg/ ml and 2.5 mg/ ml. These extracts exerted broad spectrum antifungal activity were considered for combination studies against yeasts. When the extracts were combined the FICI of the combinations LEtOH/ TwH_20, StH_20/ TwH_20, LEtOH/ BH_20 and StH_20/ BH_20 varied from 5.50 to 48.09 on the four tested yeast strains, exhibiting antagonistic interactions (FICI> 4). Overall, the combinations led to 2- 4 fold reduction of antifungal activity as compared to MICs of individual extracts. Thus, application of such combinations from different parts of *A. senegalensis* in the treatment of mycoses caused by *C. albicans, C. parapsilosis, C. krusei* and *C. neoformans* should be avoided (Bakarnga-Via *et. al.*, 2016).

The aerial parts of *Zuccagnia punctata* (Fabaceae) and *Larrea nitida* (Zygophyllaceae) were extracted with dichloromethane (DCM), and their extracts were tested alone and in combination of different ratios against *Candida albicans* and *C. glabrata* to characterize the most synergistic combinations. The results showed that three over four *Z. punctuate* / *L. nitida* fixed - ratio mixtures displayed synergistic interactions against *C. albicans.* The doses of the most synergistic mixture was 65.96 µg/ ml (ZpE 72%). On the other hand, one over four *Z. punctuate* / *L. nitida* fixed mixtures displayed synergistic interactions against *C. glabrata*. The doses of the most synergistic mixture was 168.23 µg/ ml (ZpE 27%; LnE = 73%). The study concluded

that the mixture of these plants especially at fixed doses which are most synergestic are of great interest for the development of an antifungal phytomedicine (Butassi *et. al.*, 2015).

The leaves of *Cassia alata* (Fabaceae) and *Ocimum sanctum* (Lamiaceae) were macerated separately in ethanol 95% and a stock of each extract was prepared in 5 % dimethyl sulfoxide (DMSO) in the final concentration of 2 mg/ ml. The macrobroth-dilution technique was employed for the susceptibility testing against cryptococcosis. The individual results revealed that the ethanolic extract of *O. sanctum* did not show any activity against all the strains up to a concentration of 1,000 mg/ ml (MIC) when tested alone, while the MIC of ethanolic extract of *C. alata* ranged from 500– 1,000 mg/ ml at acidic pH, the 1,000 mg/ ml concentration of the extract was found to be fungicidal in action. Furthermore, the activity of the extract was recorded to be thermolabile. The combination of both extracts inhibited the growth of the organism at a concentration ranging from 62.5– 125 mg/ ml. A 125 mg/ ml concentration of the extract combination was found to be fungicidal in action. The combination of extract was heat stable and active at acidic pH (Ranganathan and Balajee, 2000).

The study was conducted to investigate the antifungal activity of the hydro-distilled essential oil of *Foeniculum vulgare* (Apiaceae) seeds, the alcoholic extract of *Nigella sativa* (Ranunculaceae) seeds and the aqueous extract of aerial part of *Camellia sinensis* (Theaceae), they were used alone and in combination against 39 different *Candida* species, such as *C. albicans, C. tropicalis, C. krusei, C. glabrata, C. dubliniensis* and other *Candida* spp isolated from denture wearers. (Three different herbal mixtures were prepared with different concentrations as follows:

(i) *N. sativa* (20 µL) + *F. vulgare* (5 µL) + *C. sinensis* (5 µL);

(ii) *N. sativa* (15 µL) + *F. vulgare* (10 µL) + *C. sinensis* (5 µL);

(iii) *N. sativa* (10 µL) + *F. vulgare* (15 µL) + *C. sinensis* (5 µL).

The results obtained by using the punch hole method was reported as inhibition zones; with the exception of the aqueous extract of *C. sinensis*, other plants showed remarkable antifungal activity against almost all of the tested *Candida* strains. Among them, the best anti-Candidal activity was found with the alcoholic extract of *N. sativa* (mean value: 12.3 mm), followed by essential oil of *F. vulgare* (mean value: 7.9 mm); the results also exhibited that all herbal mixtures were active against various tested *Candida* isolates, ranging from 7.8 to 15 mm, 7.6 to 15.5 mm and 7 to 15 mm inhibition zones for herbal mixtures no. i, ii and iii, respectively. The highest inhibition zone was related to mixture no. ii (mean value: 12.3 mm), followed by mixture no. i (mean value: 12.1 mm) and mixture no. iii (mean value: 10.8 mm). Although lower concentrations of *N. sativa* along with higher concentrations of *F. vulgare* led to lower activity of herbal mixtures, but there were no significant differences in action of the three herbal mixtures. The highest and lowest activities of the tested mixtures were seen against *C. krusei* and *C. albicans* respectively (Naeini *et. al.*, 2017).

Aerial parts of *Baccharis glutinosa* (Asteraceae) and *Jacquinia macrocarpa* (Primulaceae) were extracted with 70% methanol. The obtained extracts were re-suspended in water and sequentially partitioned with hexane, ethyl acetate and n-butanol. The ethyl acetate fraction of *B. glutinosa* methanolic and the n-butanol fraction of *J. macrocarpa* were used in the elaboration of antifungal mixtures against

Aspergillus flavus and *Fusarium verticillioides*, because these fractions were reported previously with antifungal activity. The MIC_{50} of each fraction was determined and the Fractional Inhibitory Concentration index (FIC index) was also calculated in order to evaluate their synergistic effect. The MIC_{50} of ethyl acetate fraction of *B. glutinosa* against *A. flavus* was 1.1 mg/ ml, whereas the MIC_{50} of n-butanol fraction of *J. macrocarpa* against *F. verticillioides* was 0.3 mg/ ml, the FIC indices of mixtures that showed the highest antifungal activity against *A. flavus* and *F. verticillioides* were 0.5272 and 0.4577 respectively, indicating a synergistic effect against both fungi. The results indicated that the synergistic mixtures strongly affected the fungal growth even at lower concentrations than those of the individual plant fractions. In practice only 12% and 8% of the spores of *A. flavus* and *F. verticillioides*, respectively, treated with the synergistic mixtures, were able to germinate (Medina-López *et. al.*, 2016).

The leaves of *Monodora tenuifolia (*Annonaceae), *Terminalia catappa* and *T. mantaly* (Combretaceae) were soaked with distilled water, 70% and 95% ethanol respectively. The extracts were tested against *Candida albicans, C. glabrata* and *C. parapsilosis* by dilution method using Muller Hinton Agar. MIC and MFC were calculated. In individual assay, the results showed that extracts from *Terminalia* species were the most active with their MIC ranged between 0.0781 and 2.5 mg/ ml for *T. catappa*, and from 0.0391 to 0.3125 mg/ ml for *T. mantaly*; except for the ethanolic extract of *T. catappa* which is fungistatic on *C. glabrata*, the others extracts from this plant were fungicides on the tested yeasts. All the extracts of *T. mantaly* were fungicides on *C. glabrata* and *C. albicans.* Combinations of active sub-fractions were tested by checkerboard method with some modifications, FICI and building isobolograms methods were applied, it was concluded that there was no change in the activity of the sub-fractions from *T. catappa* in comparison with the partitionated fractions; with sub-fractions from *T. mantaly*, there were an increase in their activity compared to those of the previous fractions; the sub-fractions from *M. tenuifolia* were less active than their fractions (Jiatsa *et. al.*, 2013).

Toddalia asiatica (Rutaceae) roots, *Rhamnus staddo* (Rhamnaceae) roots, *Momordica foetida* (Cucurbitaceae) shoots, *Podocarpus falcatus* (Podocarpaceae) bark, *Aloe spp* (Asphodelaceae) succulent leaves and other individual plants were tested alone and in combinations against *Aspergillus niger* and *Candida albicans.* The plant samples were extracted by hot water, cold water and organic solvents dichloromethane/ methanol (1:1). The aqueous extracts of these plants did not show any activity when tested alone, the DCM/ methanol extracts of *P. falcatus* showed the highest activity (77.77% inhibition) against A. *niger* while *M. foetida* showed the highest activity (77.78% inhibition) against *C. albicans, Aloe spp.* showed no activity against *A. niger*. The combination of extracts (including the inactive *Aloe spp.* extract against *A. niger*) showed activity against both *C. albicans* and *A. niger*; from the results, it was clear that although the activities of individual extracts of *M. foetida, R. staddo* and *Aloe spp.* against *C. albicans* were significantly similar to that of the mixture (combined proportions), the percentage inhibitions of these individual extracts were higher when tested singly. On the other hand individual extracts of *T. asiatica* and *Aloe spp*. against *A. niger* were comparatively lower than that of the mixture. Hence a mixed actions of antagonism, additive and synergism were observed when combinations of the herbal plants were assayed (Odhiambo *et. al.*, 2009).

The stem-bark of *Euphorbia abyssinica* (Euphorbiaceae) and the whole plants of *Coleus* species (Lamiaceae) were extracted with methanol. The plant extracts were both assayed individually and in combinations (using a pour- plate method) against *Candida albicans, Trichophyton mentagrophytes, Microsporum gypseum* and *Epidermophyton floccossum*. Two assay methods were employed, they were checker board assay and time kill assay. The more potent single plant extract was *Coleus* spp. and its effect alone on *C. albicans* and *T. mentagrophy*tes cells showed that the extract at MIC and at double the MIC concentrations decreased the cell counts to about 0.05 log 10 in 48 hours; this same double MIC (15.6 mg/ ml) killed *M. gypseum* cells in 6 hours. However, when *C.* spp. and *E. abyssinica* extracts were combined together, they exhibited no synergistic interactions against *C. albicans, T. mentagrophytes* and *M. gypseum.* In 48 hours, *Coleus* spp. at MIC of 0.98 mg/ ml decreased *E. floccosum* viable cell counts from $1x10^5$ CFU to 0.97 log10; when the MIC was doubled to 1.96 mg/ ml, the *E. floccosum* cells were all killed in 3 hours. The 1 µg/ ml of the control drug inhibited the fungal cells in 48 hours When *Coleus* spp. and *E. abyssinica* extracts were combined and the activity compared to *Coleus* spp. extract alone, it was observed that the interactions showed synergistic effects against *E. floccosum* in the time kill assay, the combinations showed synergy on *E. floccossum* only. It showed additive or antagonistic activity on the rest of the tested fungi. The checker board assay method of evaluating the antifungal effects of interactions between *E. abyssinica* and *Coleus* spp. extracts showed that the combined effect was synergistic against *T. mentagrophytes*. Another synergistic effects were observed with *M. gypseum* at four different combinations of *E. abyssinica* and *Coleus* spp extracts proportions. *C. albicans* showed some significant level of antagonism to the various tested combinations (Ebob *et al.*, 2019).

The leaves of *Dissotis multiflora* (Melastomataceae) and *Paullinia pinnata* (Sapindaceae) were macerated in ethanol 95 % and assayed with Agar- well diffusion method against six fungal species, including *Candida krusei, C. tropicalis, C. parapsilosis, C. haemulinii, C. lipolytica* and *C. albicans* alongside with Flucoconazole and nystatin standards. Inhibition zone diameters, MIC and MFC were calculated. The MFC/ MIC ratio showed that the methanolic fractions of *D. multiflora* and *P. pinnata* have a fungicidal action on all 6 species of *Candida*. In general, all of six fungal strains were susceptible to different extracts and fractions with inhibition diameters ranging from 10.33 mm for methanolic fraction of *D. multiflorao*, *C. parapsilosis* to 19 mm for the same fraction on *C. haemolinii*. Both the MICs and the MFCs of actives extracts ranged respectively from 0,78 to 12,5 mg/ ml and 1,56 to 25 mg/ ml, the majority being fungicidal. The combinations showed significant antifungal activity compared to those of the fractions taken individually, especially with MICs reductions of the order to 75%. The combination of methanolic fractions of *D. multifora* and *P. pinnata* showed a synergistic effect against *C. krusei* and *C. albicans* (Ngandeu *et. al.*, 2019).

The antifungal activity of about 50 plants were tested individually and in combinations against *Fusarium oxysporum* f. sp. *Ciceris*, a causal organism of *Fusarium* wilt of chickpea. The results showed differential activity of the tested plant extracts against mycelium growth. Generally, the combined roots decoction extract of *Acacia catechu* (Mimosaceae) and leaf decoction extracts of *Lowsonia alba* (Lythraceae) (combined in ratio 1:1) showed strong enhancement in activities over

their individual use (86.42%), the percentages of mycelium growth inhibition of both *A. catechu* and *L. alba* extracts were 73.58% and 82.54% respectively (Bhardwaj and Laura, 2021).

Plant(s) – drug(s) combinations:

The *Silybum marianum* (Asteraceae) seeds were extracted with water. Then the effect of the extract was investigated both individually and in combination with fluconazole, against drug- resistant clinical isolates of *Candida albicans* and *C. glabrata*. The mean MIC_{90} of fluconazole against *C. albicans* and *C. glabrata* were determined at 512 µg/ ml and the MIC_{90} of *S. marianum* alone was 2,048 µg/ ml. The MIC_{90} of *S. marianum* extract in combination with fluconazole was found to be 128 µg/ ml, four times lower than that of fluconazole (MIC_{90} = 512 µg/ ml) and 16 times lower than that of the extract individually. The aqueous extract of *S. marianum* in combination with fluconazole was found to have a more potent *in vitro* activity than the extract and drug individually (Fozouni and Palang, 2018).

The leaves, twigs and stem of plants *Uvaria angolensis, U. muricata* (Annonaceae) and *Terminalia catappa* (Combretaceae) were extracted by water and ethanol. The extracts were evaluated and their optimization with nystatin and ketoconazole was done against yeasts species by using agar dilution method. Broth micro dilution method and subculture were used to determine their antifungal parameters (MIC and MFC). The results showed that the leaves extract of *T. catappa* showed the best antifungal activity with MIC of 1.56 mg/ ml, 0.78 mg/ ml and 0.78 mg/ ml respectively on *Candida albicans, Cryptococcus neoformans* and *Candida parapsilosis* respectively isolated from HIV patients. The interaction study of the combination of the promising extracts with nystatin and ketoconazole presented synergistic effects with the best index being FIC Index of 0.17 ± 0.09 from *T. catappa* extract on *C. albicans* and a significant reduction of the MIC values of the extracts, nystatin (3 to 1,600 times) and ketoconazole (2 to 512 times); these synergistic results support the traditional use of these plants in the treatment of infectious diseases and suggest that they could serve as potential sources of antifungal (Toghueo and Boyom, 2014).

A concentrated extract of garlic (*Allium sativum*, Liliaceae) was prepared with ethyl acetate. Minimal inhibitory concentrations (MIC) of *A. sativum* extract and amphotericin B were determined by a broth dilution technique method, against three clinical isolates of *Cryptococcus neoformans.* It was found that the concentrated extract of *A. sativum* possessed potent *in vitro* fungistatic and fungicidal activities against the three isolates of *C. neoformans*, the MIC of the garlic extract was raging between 6.1– 12.2 µg/ ml, while the MIC of amphotericin B against the three isolates was ranging between 0.1– 0.2 µg/ ml. The MFC was 12.2 µg/ ml and 0.2– 0.4 µg/ml for concentrated garlic extract and amphotericin B respectively. In combination study, the isobolograms demonstrated that amphotericin B had synergistic antifungal activity with the concentrated garlic extract, the fractional inhibitory concentration indices were 0.5. (synergism). The level of synergy was comparable to that combination of amphotericin B and flucytosine (Davis *et. al.*, 1994).

Astronium urundeuva (Anacardiaceae) leaves were exhaustively percolated by hydro-ethanol. *In vitro* susceptibility test to examine the anti-fungal activity was carried,

against collected strains and clinical isolates of *Candida albicans* and *C. glabrata*, followed by determination of minimum fungicidal concentration (MIC) and finally determination of the activity of the free extract in combination with clinically used anti-fungal agents. Individual *in vitro* susceptibility assays for each drug alone demonstrated the anti-fungal activity of the free extract against both *Candida* species, with increased activity against *C. glabrata*, including collected strains and clinical isolates displaying different levels of resistance against the most common clinically used anti-fungal drugs. In the checkerboard assays for combination study, different concentrations of the free extract were used in combination with different dilutions of fluconazole, caspofungin and amphotericin B, both against *C. albicans* and *C. glabrata*, a majority of combinations showed indifference, with the notable exemption of the combinations between the free extract and amphotericin B against *C. albicans*, which resulted in synergism. Therefore, if this plant extract could be used combined with anti-fungal agent, both at lower concentrations, it could lead to increased activity and reduces the negative side effects of amphotericin B, that is nephrotoxicity (Bonifácio *et. al.*, 2019).

A flower of *Flos Rosa Chinensis* (Rosaceae) was extracted with ethanol 70% to investigate the anti-fungal activity of the hydro-alcoholic extract of *R. Chinensis* (FRC) combined with fluconazole against thirteen clinical isolates of *Candida albicans* resistant strains to flucoconazole. The minimum inhibitory concentration (MIC) of the extract was determined using a checkerboard micro dilution assay. *R. chinensis* alone exerted efficient antifungal activities against *C. albicans* within a MIC_{80} ranging from 20 μg/ ml to 40 μg/ ml, the plant extract failed to enhance the effects of fluconazole against sensitive *C. albicans* strains, although it rendered fluconazole-resistant *C. albicans* more sensitive. By in vivo studies, the *R. chinensis* antifungal mechanism showed that it strengthens fluconazole to inhibit the action of ergosterol biosynthesis by promoting the transformation of lanosterol to eburicol, suggesting that the antifungal mechanism of action involves the inhibition of ergosterol biosynthesis (Zhang *et. al.*, 2017).

In vitro anti-fungal activity of fractions obtained from the lyophilized boiled infused aqueous extract of the leaves of *Acca sellowiana* (Myrtaceae) was evaluated against resistant strains of non-albicans *Candida* (NAC). Its reversal of fluconazole (FLZ) resistance was also evaluated by combining it with the drug. The anti-fungal activity of the fractions (F1, F2 and F3) was evaluated at 500 µg/ ml by micro dilution method. *C. glabrata* showed the lowest MIC values (500– 3.90 µg/ ml), and among all fractions the F2 active fraction was the most effective. Checkerboard assay was performed to determine the effect of the combination of the F2 fraction and FLZ, the association of F2 with FLZ produced FICI of ≤ 0.5 (synergism) against 100% of *C. glabrata* resistant isolates. This study suggests that the combination of F2 active fraction and FLZ might be used as an alternative treatment for mucocutaneus infections caused by resistant strains of NAC (Machado *et. al.*, 2016).

Uncaria tomentosa (Rubiaceae) stem barks were extracted with hydro-ethanolic solutions 50% (v/v) for the *in vitro* synergism test of its water insoluble fraction (WIF) in combination with fluconazole and terbinafine (FLZ and TRB), against resistant non *Candida albicans* isolates. FLZ and TRB alone and combined with WIF were tested by the checkerboard method using the micro-dilution technique, *Candida*

krusei ATCC 6258, CK01, CK04 and *Candida glabrata* CG40039, CG10, RL02, RL03 were tested. The results indicate that TRB and FLZ tested alone up to a 64 µg/ ml was unable to inhibit the growth of any isolated organism strains. The addition of WIF to TRB resulted in enhancement of the anti-fungal activity of TRB, so TRB and WIF combined in a 8:1.95 µg/ ml concentration ratio caused higher growth inhibition on the CK6258 terbinafine resistant isolate (88%) than TRB and WIF alone (40.7% and 20% respectively); TRB and WIF at 4:1.95 µg/ ml concentration ratio caused significant cell damage (79.52%) regarding the CK04 isolate; TRB and WIF combination was able to induce a significant synergic effect on near all isolates. Regarding the FLZ resistant isolate of CK04, a cell damage of about 80% could be noticed for TRB and WIF combined in a concentration ratio of 1.95:8 µg/ ml; in that case, the compounds alone showed 50% of cell damage below using the same concentration ratio. The combination of WIF with TRB and FLZ was able to reduce the MIC values determined by the inhibitory assay and either additive or synergic effects could be clearly noticed in all tested isolates. For TRB and WIF combination a synergistic effect was observed in four different isolates, being two *C. krusei* and two *C. glabrata*; but also for WIF and FLZ combination in three isolates, being one *C. krusei* and two *C. glabrata*. In other isolates an additive effect was observed (Moraes *et. al.*, 2017).

Absolute ethanol was used to prepare an extract of dried *Ocimum basilicum* (Lamiaceae) leaves, the extract was suspended in distilled water and fractioned successively with n-hexane, dichloromethane, ethyl acetate and n-butanol. The anti-cryptococcal activity of ethanol crude extract and hexane fraction and its synergism with amphotericin B (AMB) and *O. basilicum* essential oil (EO) was conducted against three clinical strains of *Cryptococcus neoformans* T444, *C. neoformans* H99 and *C. gattii* WM779; all the combinations tested produced FIC index values ranging from 0.187 to 0.75, this showed that all these combinations reduced the MIC values. The synergistic effect was observed in the combination of amphotericin B and ethanol crude extract, reducing their MIC from 1.56 to 0.099 µg/ ml and 625 to 78 µg/ ml respectively; in the combination of ethanol crude extract with essential oil, it was observed that there was reduction in their MIC values from 625 to 39 µg/ ml and 1,250 to 157.2 µg/ ml respectively, and in the combination of hexane fraction and essential oil, it was observed a reduction in their MIC from 156 to 20 µg/ ml and 1,250 to 78.72 µg/ ml respectively. When amphotericin B was combined with 78 µg/ ml with hexane fraction, their MIC values were reduced from 1.56 to 0.396. Most of combination resulted in synergistic effect. Only the combination of amphotericin B with hexane fraction result in an additive effect (Cardoso *et. al.*, 2017).

Leaves of *Eugenia uniflora* (Myrtaceae) were extracted by maceration with 95% ethanol. The *E. uniflora* ethanol extract was diluted by DMSO, the extract was assayed for its anti-fungal activity, either alone or combined with four selected chemotherapeutic antimicrobial agents, including amphotericin B, mebendazole, nystatin and metronidazole against *Candida albicans*, *C. krusei* and *C. tropicalis* ATCC 13803. The MIC was >1,024 µg/ ml, which did not demonstrate clinical relevance of the possible use of the *E. uniflora* ethanol extract as an anti-fungal drug. However, an interesting potentiation of the anti-fungal activity was demonstrated when *E. uniflora* ethanol extract was associated with metronidazole against the *C. tropicalis* strain, lowering the MIC of this anti-fungal drug fourfold (from 128 to 32),

the obtained results indicated that the association of the extract of *E. uniflora* to metronidazole showed a potential anti-fungal activity against *C. tropicalis*. However, no synergistic activity against the other strains was observed. The study indicates that *E. uniflora* (and the family Myrtaceae in general) could be a source of nutraceuticals with an antifungal-modifying activity, representing an interesting alternative to combat infectious diseases such as candidiasis. This plant appears to be promising in the development of therapies, mainly due to its low toxicity *in vitro*, which allows to proceed with *in vivo* studies for drug evaluation (Santos *et. al.*, 2013).

Rubus chingii (Rosaceae) fruit powder was extracted with 70% ethanol and investigated for the anti-fungal activity in combination with fluconazole (FLC) against fluconazole-resistant *Candida albicans* (*in vitro*). *R. chingii* extract and fluconazole resistant *C. albicans* fungal suspension were prepared, the minimum inhibitory concentration (MIC) and fractional inhibitory concentration index (FICI) of *R. chingii* extract combined with FLC against *C. albicans* were determined, after which the growth curves for *C. albicans* treated with *R. chingii* extract, FLC alone and a combination of these preparations were constructed. Although neither *R. chingii* extract nor FLC alone showed obvious anti-fungal activity, the two drugs together showed significant synergy. The MIC80 for FLC alone was >256 mg/ ml and for *R. chingii* extract alone was >5,000 mg/ ml. However, the MIC80 for the two drugs combined was only 0.0625– 16 mg/ ml for FLC and 4.88– 312.5 mg/ ml for *R. chingii* extract. Reduction in MIC values proof that the interaction was synergistic (Han *et. al.*, 2016).

The aerial parts of *Sarcococca saligna* (Buxaceae) were percolated with 95% ethanol. A disk diffusion method was used to assay the anti-fungal activity of *S. saligna* ethanolic extract and its combination effect with fluconazole against clinical test strains of *Aspergillus* Species (*A. niger, A. treus, A. flavus and A. Fumigates*) on Sabouraud dextrose agar. The activity was measured in form of zone of inhibition. No clear zones of inhibition were observed for all test strains around standard fluconazole paper disks, and this confirmed that these test strains were resistant to fluconazole. The *S. saligna* extract showed anti-fungal activity (MIC) at content of $\geq$ 0.5 mg/ disk against *A. niger* and *A. treus*. The anti-fungal activity of the plant extract against the mentioned test strains was dose-dependent and increased with the increase in the plant extract concentrations. In contrast, S. *saligna* extract did not show anti-fungal activity against *A. flavus* at contents used for the bioassay (0.5, 1, 2, 3 and 4 mg/ disk). Furthermore, another tested strain (*A. fumigates*) was less susceptible to *S. saligna* extract compared with *A. niger* and *A. treus*. No inhibition zones were observed for *A. fumigates* at lowest contents of *S. saligna* ethanol extract (0.5, 1 and 2 mg/ disk). The combination effect of this plant extract at the same amounts (0.5, 1, 2, 3 and 4 mg/ disk) with fluconazole (25 µg/ disk) was also investigated against the mentioned *Aspergillus* species and has been reported, the ethanol extract of *S. saligna* enhanced the anti-fungal activity of fluconazole against *A. niger, A. treus* and *A. flavus*. At the highest tested contents of 4 mg/ disk, 1.15-, 0.64- and 2.47- fold increases in inhibition zone surface area were observed for *A. niger, A. treus* and *A. flavus* respectively which indicate synergism. However, no enhancing effect was observed by the plant extract against *A. fumigates* at tested concentrations of the extract (Moghaddam *et. al.*, 2009).

Hippophae rhamnoides (Elaeagnaceae) twigs and leaves were each extracted with 80% methanol. The extracts were then *in vitro* studied in terms of anti-candidal activity of each extract alone and in combination with either fluconazole (FLC) or caspofungin. The MIC were determined using two different methods (micro dilution broth assay and agar dilution assay); in both methods *H. rhamnoides* extracts were initially dissolved in 50% DMSO. The MIC values of the twigs and leaves extracts against *C. albicans* ATCC 10231 were established as 250 mg/ ml and 31.5 mg/ ml respectively, unexpectedly, the growth of the clinical *C. glabrata* G1 strain (blood isolate) included to this stage of the study was inhibited by the twigs extract at a relatively low concentration of 15.6 mg/ ml and by the leaves extract at a concentration as low as 3.9 mg/ ml; in the combination study the findings indicate that the MIC values of FLC and CAS were decreased by the co-action of the extracts, these extract preparations increased the fungistatic action of FLC against both *C. albicans* and *C. glabrata* which conclude *H. rhamnoides* exhibit significant anti-fungal activity and affects important Candida virulence factors, thus have a good potential for the development of novel antifungal products supporting classic drugs (Sadowska *et. al.*, 2017).

The dried fruits of *Terminalia chebula* (Combretaceae) were extracted with methanol. *In vitro* anti-fungal activity of the crude extract was studied against *Candida albicans* by the agar well diffusion method. The combination of the plant extract with amphotericin B was also studied. The plant extract and amphotericin B were dissolved in DMSO. In case of the combination, equal volume (25µl) of each was added in the well and zone of inhibition was measured. The results revealed that the plant extract had not showed inhibition at concentration of 10 mg/ ml and 30 mg/ ml but at the same concentration amphotericin B showed susceptibility against *C. albicans*. When the plant extract combined with amphotericin B the zone of inhibition had been increased significantly. The enhancement of anti-fungal activity in case of combination of plant extract and amphotericin B could be explained by the presence of biologically active compounds which are present in the plant extract. Thus, combination of *T. chebula* extract with amphotericin B could be beneficial to increase anti-fungal activity against *C. albicans* (Vyas *et. al.*, 2014).

Seeds powder of *Pimpinella anisum* (Apiaceae) and *Moringa oleifera* (Moringaceae) leaves were extracted separately with distilled water. 250 µg/ ml of terbinafine (anti-fungal agent) was dissolved in DMSO, MIC of the individual drug and its combination with plant extracts were calculated against the pathogenic *Microsporum canis*. Terbinafine has a MIC of 6 µg /ml, whereas *M. oleifera* and *P. anisum* extracts have a MIC of 80 mg/ ml and 60 mg/ ml, respectively. A combination of terbinafine, *M. oleifera* and *P. anisum* had the greatest effect in inhibiting the development of the pathogenic fungi. In comparison to the control experiment, all combinations were found to have a considerable impact on the growth of *M. canis* throughout the experiment. In addition, all the treatments comprising terbinafine and a plant extract has a higher inhibitory effect compared to combination of plant extracts (*M. oleifera* in combination with *P. anisum*) treatments and the control experiment. This implies that the terbinafine was more effective in inhibiting fungal growth when used in a combination treatment with plant extracts (Khazia and Al-Janabi, 2019).

Vernonia adoensis (Asteraceae) leaves were extracted by hexane, dichloromethane, ethyl acetate, dichloromethane/ Methanol, ethanol, methanol and water. The extracts were tested for their potency on inhibiting the growth of *Candida krusei.* The MICs were determined from the most potent extract. The effects of combining fluconazole and the most potent extract were also investigated. The result for the effect of drug alone showed the MIC of fluconazole for *C. albicans* was found to be 8 µg/ ml while the MIC for *C. krusei* was 125 µg/ ml which is higher than that of *C. albicans*. The MFC for fluconazole on C. *krusei* was also 125 µg/ ml. Thus, *C. krusei*, which is intrinsically resistant to fluconazole, was found to be less sensitive to the effects of fluconazole when compared to *C. albicans*. Subsequent work was then conducted on *C. krusei* only. The result for effect of plant extracts imply that all extracts (except water extract) had no effect on inhibiting the growth of *C. krusei*, as the cell densities were high. Significant potency was generally shown for the highest concentrations although there was no MIC. Distilled water extract significantly reduced the growth of the fungi, it was combined with fluconazole to determine if there was enhanced effects, concentrations of fluconazole were ranged from 500 µg/ ml to 8µg/ ml, and were combined with the water extract from 100 µg/ ml to 12.5 µg/ ml, MIC of the combination of 100 µg/ ml of the water extract and 32 µg/ ml of fluconazole was obtained, the combination lowered the MIC of fluconazole on *C. krusei* from 125 µg/ ml to 32 µg/ ml, as the water extracts concentrations increased, the cell densities decreased with a greater decrease observed at higher concentrations of fluconazole. Combining different concentrations of fluconazole with 100 µg/ ml of the water extract increased the potency of fluconazole (Nyamuriya *et. al*., 2018).

Allium sativum (Alliaceae) and *Nigella sativa* (Ranunculaceae) were tested together and each one alone with fluconazole against *Candida albicans*, the first plant was extracted with distilled water while the second plant was extracted by ethanol. The results indicated that *N. sativa* alone does not produce an inhibition zone (up to 10% concentration) and when combined with *A. sativum* prompted decreasing in the size of the inhibition zones against *C. albicans* compared to *A. sativum* alone. Again, *A. sativum* extract with *N. sativa* might prompt an increasing in the size of the inhibition zone against *C. albicans* compared to fluconazole alone. The fungicidal activity of *N. sativa* increased by *A. sativum* extract, and the synergism *N. sativa* with *A. sativum* extract produce more zone (0.996 mm) compared fluconazole alone (0.833 mm) respectively. This is to say that *A. sativum* extract has effects on *C. albicans, N. sativa* doesn't has effects on *C. albicans*, synergistic of *N. sativa* extract with *A. sativum* extract have less effect than *A. sativum* extract alone but more effect than fluconazole alone (Salih, 2016).

A randomized controlled clinical trial was applied to determine the effect of *Salvia officinalis* (Lamiaceae) extract as vaginal tablets alone and in combination with clotrimazole, on the recovery of vulvo-vaginal candidiasis and to compare its effectiveness. 111 participants were randomly assigned into three groups of 37 patients using block randomization with block sizes of 6 and 9, and allocation ratio of 1:1:1: 100 mg vaginal tablet of clotrimazole and placebo (CP), 400 mg vaginal tablet of *S. officinalis* and placebo (SP), and vaginal tablet of *S. officinali*s and clotrimazole (SC), once daily for 7 days. On the seventh day after the treatment was ended up, vulvo-vaginal candidiasis were examined by vaginal symptoms and wet test, and if positive, they were examined by culture in chrome agar *Candida* medium. The

frequency of a positive wet test confirmed by sabrodextrose agar medium 7 days after treatment was significantly lower in the group taking *S. officinalis* and clotrimazole than the reference group of *S. officinalis* and placebo. There was no significant difference in the group taking placebo with either *S. officinalis* or clotrimazole. This made conclusion that *S. officinalis* in the form of vaginal tablet, alone and when combined with clotrimazole, can treat the vulvo-vaginal candidiasis (Ahangari *et. al.*, 2019).

Acmella caulirhiza (Asteraceae) and *Senna didymobotrya* (Fabaceae) extracts were tested against *Candida* spp., hexane and methanol extracts were prepared by maceration from each extract. Clotrimazole, ketoconazole, nystatin, amphotericin B and griseofulvin were dissolved in DMSO. Methanol and hexane extracts of *A. caulirhiza* and *S. didymobotrya* were also weighed and dissolved in DMSO to give stock solutions. The test organisms (*Candida* spp.) that were used are: *Candida albicans, C. duabushaemulonii, C. haemulonii, C. auris, C. famata, C. orientaris* and *C. krusei*. MIC values were determined by broth micro-dilution test and was found that griseofulvin, clotrimazole and ketoconazole produced high MIC values in comparison to the control drug; for nystatin, however, the results were erratic with growth at low concentration and no zone of inhibition at higher concentrations meaning that all the pathogens died. From the plant extract/ conventional drug concentration gradients most of the combinations showed MIC values at lower conventional drug concentration and higher extract concentration; two combinations, however, amphotericin B/ *A. caulirhiza* methanol extract and ketoconazole/ *S. didymobotrya* hexane extract deviated from this observation where the extract concentration was lower than the conventional drug concentration; amphotericin B/ *A. caulirhiza* hexane extract combination was synergistic when used against *C. krusei* and *C. orientaris*; while with the other *Candida* species it was antagonistic. Clotrimazole/ *A. caulirhiza* hexane extract combination was synergistic against *C. albicans, C. krusei* and *C. orientaris* but antagonistic against the other *Candida* species. The other combinations were indifferent and antagonistic against the *Candida* species used. This study found that *A. caulirhiza* and *S. didymobotrya* have potent anti-fungal phytochemicals and thus *A. caulirhiza* extract modulates clotrimazole. It is thus recommended that pure active anti-fungal components of these plants be determined and pure active components of *A. caulirhiza* be used to develop new anti-fungal regimens in combination with clotrimazole (Olwenya *et. al.*, 2019).

Dried aerial parts of *Echinophora platyloba* (Apiaceae) were extracted by maceration with 70% ethanol. Three different concentrations of the ethanolic extracts (4, 5.2, and 11%) were prepared. The antimicrobial and anti-fungal activities of each extract alone was evaluated against dermatophytes, *Candida albicans* by agar dilution and micro broth dilution assays against many diseases causing agents including *Candida albicans*. The susceptibility of *C. albicans* (MIC and MLC) and the corresponding size of zone of inhibition to different types and concentrations of *E. platyloba* and amphotericin B each one alone and in combination by disc diffusion method were observed. *C. albicans* growth was inhibited by concentrations $\geq$ 2 mg/ ml of extract (2, 4, 8, 16, 32, 64, 128 and 256 mg/ ml), there was a 50% reduction in MIC and a 75% reduction in MLC values of the mixture of amphotericin B and 5% ethanolic extract against *C. albicans* in comparison to amphotericin B alone; the zone of inhibition of the mixture showed 22% increase in diameter in comparison to that of

amphotericin B alone. In this test, the most potent anti-fungal agent was the mixture of ethanolic extract 5% plus amphotericin B, followed by amphotericin B, ethanolic extract 5%, ethanolic extract 11%, ethanolic extract 4% and ethanol 70% in descending order. Also, the results showed that 5% ethanolic extract was slightly stronger than 11% ethanolic extract. Regarding this study, it is clear that *E. platyloba* indeed exhibits a potent anti-fungal activity. Its inhibitory action against *C. albicans* was the highest and some degrees of synergy was recorded in combination of amphotericin B plus *E. platyloba* 5% ethanolic extract covering *C. albicans*. The synergistic combined mixture in this *in vitro* study need further *in vivo* studies to evaluate its actual effect (Majid *et. al.*, 2010).

The effects of the aqueous and methanol extracts of green tea leaves (*Camellia sinensis*, Theaceae) and the synergistic effects of these two extracts were studied along with two drugs of itraconazole and voriconazole against four strains of *Aspergillus* species. Micro dilution method was used to determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). The results concluded that two strains of *A. flavus* and *A. terreus* were sensitive to itraconazole while two other strains were resistant; all four tested strains were resistant to voriconazole. The aqueous and methanol extracts of green tea did not show anti-fungal activity when tested individually, but synergistic effects of aqueous extract of green tea and itraconazole were worthwhile against *A. niger* and *A. fumigatus*. Also, the combined methanol extracts of green tea and itraconazole against *A. niger* and the combined aqueous extracts of green tea and voriconazole against *A. flavus* and *A. fumigatus* were reported valuable. The results of this study showed that there is a valuable synergistic effect between the tested anti-fungal drugs and the extracts (Sarkhani Moghaddam *et. al.*, 2018).

In the study to find the effect of *Hibiscus sabdariffa* (Malvaceae) roselle extract in synergism with voriconazole and fluconazole against fluconazole- resistant *Candida albicans* isolates, air- dried calyces were extracted using 80% hydo- methanol. Determination were done by plate antimicrobial susceptibility testing method. Checkerboard assays were applied to find the interaction of *H. sabdariffa* extract with fluconazole and voriconazole. Individual antimicrobial activity showed various levels of MIC for the extract were observed against all the isolates. MIC values ranged from 0.5 to 2 mg/ ml. All the isolates showed resistance to fluconazole (MIC > 16 µg/ml). All the isolates showed susceptibility to voriconazole with MICs values of < 0.016 µg/ ml. The results from the checkerboard assay indicate that the combinations of fluconazole and the extract have an ‘indifference’ effect; in general, the checkerboard results indicate that combinations of fluconazole and *H. sabdariffa* have no synergism except for one strain among the six tested strains. In case of variconazole, the extract was associated with voriconazole at different concentrations and there was a reduction of the voriconazole MICs, in almost all of the six strains tested for combination of extract and variconazole, there was a strong synergistic effect demonstrated by the FICI calculation (Alshami and Alharbi, 2014).

The aerial parts of the six selected plants [i.e. *Atriplex halimus* (Amaranthaceae), *Alhagi maurorum* (Fabaceae), *Brassica tournefortii* (Brassicaceae), *Nicotiana glauca* (Solanaceae), *Mesembryanthemum crystallinum* (Aizoaceae) and *Peganum harmala* (Zygophyllaceae)] were extracted with aqueous and other organic solvents (i.e.

petroleum ether, chloroform, ethyl acetate and methanol). Then they were screened against the different human pathogenic fungal species (*Candida albicans, C. tropicalis*, *Trichosporon* spp, *Aspergillus fumigatus, A. flavus* and *A. versicolor*) alone and in combination between them. The results indicate that petroleum ether fractions of *M. crystallinum, N. glauca, P. harmala*, *A. halimus* and *B. tournefortii* were the most active fractions compared with chloroform, ethyl acetate and methanol fractions and also with crude ethanolic extracts. The MIC values of fractions against yeasts and moulds ranged from 0.195 to 6.25mg/ ml, whereas the fungicidal activity ranged from 0.781– 12.5 mg/ ml. The most efficient anti-fungal activity was displayed by the petroleum ether fraction of *M. crystallinum* which inhibited the growth of yeast at MIC value of 0.195 mg/ ml and moulds at MIC values ranged from 1.56– 3.12 mg/ ml. The synergistic effect of most active plant fractions and fluconazole were tested using well diffusion assay, notably, the majority of combinations between one plant extracts and other plant extracts showed synergistic anti-fungal activities against the tested fungal species (for example petroleum ether fraction of *B. tournefortii* and *A. halimus*); also the results indicate that the majority of combinations between plant extracts and fluconazole showed highly synergistic effect against all tested fungal species except the combination between *A. maurorum* extract and fluconazole which showed strong antagonistic effect against *A. fumigatus* and *A. versicolor*; there was also a noticeable synergistic effect observed against *C. albicans* and *C. tropicalis*, a slight synergistic effect with *Tricosporon* spp and *A. versicolor* and antagonistic effect with *A. flavus* (Ibrahim *et. al*., 2018).

The fresh leaves of *Chromolaena odorata* (Asteraceae) were macerated in chloroform. The chloroform extract was individually evaluated for its anti-fungal activity against *Aspergillus niger* using agar cup diffusion technique; while checkerboard technique was applied for evaluation of interaction and effect of combination with itraconazole. The results revealed that *A. niger* was susceptible to itraconazole (MIC= 0.0002 mg/ ml) and also susceptible to chloroform extract of *C. odorata* (MIC= 5 mg/ ml). Concomitant administration of the extract and itraconazole drug revealed that some ratios showed additive properties, some other ratios were synergistic, while some showed indifferent activities; the combined effects of *C. odorata* leaf extract and itraconazole against *A. niger* reveal the synergistic effect was obtained in 4 ratios (9:1, 8:2, 7:3 and 5:5), with the ratio 5:5 producing the most synergistic effect. In the drug ratio that showed the greatest synergy (5:5), the MIC of itraconazole and *C. odorata* extract were reduced by 20 and 8 times respectively. The implication is that *C. odorata* extract modifies the activity of itraconazole to a much larger extent than itraconazole does alone (Ohadoma *et. al.*, 2016).

The aerial part of *Tanacetum vulgare* (Asteraceae) was extracted by ethyl acetate at room temperature. The anti-fungal effects of the ethyl acetate extract, chlorhexidine and sodium hypochlorite were tasted each one alone and in combination between them against *Candida albicans.* The results was presented in term of zone of inhibition. The inhibition zone of chlorhexidine against *C. albicans* was 30.3– 19.3 mm, but in combination with ethyl acetate extract (100 mg/ ml) of *T. vulgare* the inhibition was from 32.7– 30 mm, indicating that this combination exerted a marked synergistic effect against *C. albicans*. The inhibition zone of sodium hypochlorite (69.7– 65 mm) was higher than the inhibition zones of ethyl acetate extract and

chlorhexidine. The combination of ethyl acetate extract with sodium hypochlorite resulted in a loss of anti-fungal activity (Kameri *et. al.*, 2019).

The combination of antifungal creams with natural products from plants was a strategy to combat resistance of some antifungal agents against some fungal dermal infections, so this study revealed the antifungal effect of combined antifungal creams (clotrimazole, fluconazole, ketoconazole and terbinafine) with turmeric rhizomes essential oil (*Curcuma longa*, Zingiberaceae) or *Aloe vera* (Asphodelaceae) gel, the tested fungal species were *Candida albicans*, *Penicillum notatum, Aspergillus fumigates, A. niger, A. flavus, Trichophyton rubrum, T. violceum* and *T. mentagrophytes*. The antifungal activity was carried out using agar well diffusion method. GC-MS was applied to know the phytochemical constituents in both extracts, it showed 36 and 18 bioactive compounds in *C. longa* essential oil and *Aloe vera* gel respectively, these phytochemical compounds were related to phenols, flavonoids, saponins, alkaloids, steroids, terpenoids and cardiac glycosides. All antifungal creams applied displayed zones of inhibition with values ranged from 5 to 14.3 mm, the turmeric essential oil alone was 5 to 11 mm, while *Aloe vera* gel alone was ranged from 8 to 11.7 mm; the MIC by antifungal creams, turmeric essential oil and *Aloe vera* gel was ranged from 1.25 to 10 mg/ml. the combination of antifungal creams with turmeric essential oil or Aloe vera gel revealed synergistic and indifferent properties, so clotrimazole + turmeric E.O. against *C. albicans*, ketoconazole + turmeric E.O. against *A. niger*, terbinafine + turmeric E.O. against *C. albicans*, clotrimazole + *Aloe vera* gel against *C. albicans*, fluconazole + turmeric E.O. against *A. flavus* and terbinafine + *Aloe vera* gel against *C. tropicalis* displayed synergistic properties, while others were indifferent without antagonism (Ogidi *et.al*, 2021).

Essential oil(s) – drug(s) combinations:

Essential oils obtained from *Cinnamomum cassia* (Lauraceae), *Melaleuca alternifolia* (Myrtaceae), *Mentha piperita* (Lamiaceae), *Origanum vulgare* (Lamiaceae) and *Syzygium aromaticum* (Myrtaceae) were tested against 19 strains of *Malassezia pachydermatis* isolated from healthy dogs. The anti-fungal activity was determined by checkerboard assay to search for interactions between these essential oils and some known anti-fungal drugs; the combination concentrations of clotrimazole used was ranging from 0.0625 µg/ ml to 32 µg/ ml, while the essential oils concentrations used were as follow: *C. cassia* (0.156- 20 mg/ ml), *S. aromaticum* (0.156- 20 mg/ ml), *M. piperita* (0.4- 50 mg/ ml), *O. vulgare* (0.4 - 50 mg/ ml) and *M. alternifolia* (0.4- 50 mg/ ml). The fractional inhibitory concentration indices (FICI) of clotrimazole combined with selected essential oils were calculated. Synergism was observed for the combination of clotrimazole with *M. alternifolia*, *M. piperita* and *O. vulgare* essential oils. The combinations of *C. cassia* and *S. aromaticum* essential oils with clotrimazole showed indifferent effect. Additive effect was observed for the combination of clotrimazole with *S. aromaticum* and *M. alternifolia* essential oils against reference strain (Bohmova *et. al.*, 2019).

An essential oil from *Myrtus communis* (Myrtaceae) was evaluated for anti-fungal activity alone and in combination with amphotericin B against *Candida albicans* and different species of *Aspergillus* spp (*A. niger, A.* parasiticus & six isolates of *A. flavus*) using broth micro dilution assay. MIC and MLC values of amphotericin B were 1- 2 and 2 mg/ ml for *C. albicans* and 4- 8 and 8 mg/ ml for *Aspergillus* spp

respectively. The MICs of the tested essential oil did not differ significantly with regard to *C. albicans* and *Aspergillus* spp, the MIC and MLC values of the essential oil ranged from 8- 16 and 16- 32 ml/ ml against tested fungi; the MIC values showed that the oil was quite active against fungi at these concentrations. To explore the possibility of developing a more powerful combination therapy of the oil with amphotericin B, the checkboard micro titer test was performed, the MIC of amphotericin B alone against *C. albicans* was lowered from 2 to 0.06 mg/ ml when the essential oil was added at concentration 4 ml/ ml; the FIC of the oil in combination with amphotericin B against *C. albicans* were 0.25 ml/ ml and 0.03 mg/ ml for amphotericin B; the FIC of the oil in combination with amphotericin B against *A. niger* was 0.25 ml/ ml and 0.015 mg/ ml respectively, the FIC index showed marked synergism of the oil and amphotericin B combination against *C. albicans* and *A. niger*. As concluded the antifungal evaluating showed that essential oil of *M. communis* exhibited good anti-fungal activity against tested fungi, also it showed significant synergistic anti-fungal activity when combined with amphotericin B (Mahboubi and Bidgoli, 2010).

The study was conducted to screen certain plant essential oils [*Mentha piperita* (Lamiaceae), *Carum copticum* (Apiaceae), *Cinnamomum verum* (Lauraceae), *Syzygium aromaticum* (Myrtaceae), *Cymbopogon martini* (Poaceae) and *Thymus vulgaris* (Lamiaceae)] and to test their active compounds (such a as thymol, cinnamaldehyde, eugenol and geraniol) individually, also their *in vitro* interaction with fluconazole against drug-resistant pathogenic fungi including (*Aspergillus niger, A. fumigatus, A. solani* and *Trichophyton rubrum*) was tested. All tested essential oils exhibited strong inhibitory activity against the test strains (MIC range: 72– 288 µg/ ml; MFC range: 144– 576 µg/ ml). Oil of *M. piperita* was strongly active against *A. fumigatus* and moderately active against *T. rubrum* (MIC of 288 and 576 µg/ ml respectively). Active compounds, namely, cinnamaldehyde, eugenol and geraniol, showed activity higher than essential oils with MICs ranging from 40 to 160 µg/ ml and MFCs ranging from 80 to 320 µg/ ml; cinnamaldehyde was the most active against *A. solani* and *T. rubrum* with MICs and MFCs of 40 and 80 µg/ ml respectively. In combination study, all the tested essential oils and active compounds showed significant levels of synergistic interaction with fluconazole against *T. rubrum*, the essential oils of *S. aromaticum* (0.250), eugenol (0.375) and cinnamaldehyde (0.187) exhibited synergistic interactions with fluconazole against *A. fumigatus* but no interactions were observed for the oils of *C. martini* and geraniol with fluconazole, the maximum level of synergy was determined between cinnamaldehyde and fluconazole against both *T. rubrum* (0.156) and *A. fumigatus* (0.187). Cinnamaldehyde was the most effective in combination therapy, showing the strongest synergy with fluconazole and reducing the MIC of fluconazole up to 8 fold against both *A. fumigatus* and *T. rubrum* and a reduction in its own MIC up to 16 and 32 fold respectively. The highest reduction in MIC (i.e. 128 fold) was recorded for oil of *S. aromaticum* in combination with fluconazole against *T. rubrum*. No combination was found to be antagonistic against the tested fungi (Khan and Ahmad, 2011).

A study was conducted to evaluate the activity of fluconazole against 32 clinical strains of fluconazole- resistant *Candida albicans* after their exposure to sub-lethal concentrations of tea tree oil (TTO) distilled from specially selected *Melaleuca alternifolia* (Myrtaceae) leaves or its main bioactive component terpinen-4-ol using

broth macro dilution. *C. albicans* strains tested to fluconazole alone were resistant but the same strains were susceptible to low concentrations of TTO. The MIC values of fluconazole for the 32 clinical *C. albicans* strains ranged from 64 to 256 μg/ ml (average= 244 ± 47.22 μg/ ml). The TTO MICs for the 32 clinical *C. albicans* strains ranged from 0.06% to 0.5% (average= 0.19 ± 0.09%). Exposure of fluconazole-resistant *C. albicans* strains for 24h to 1/4 MIC (sub-lethal concentrations) of TTO and fluconazole (in combination) enhanced fluconazole activity against these strains. Overall, 62.5% of isolates were classified as susceptible, 25% exhibited intermediate susceptibility and 12.5% were resistant. For all of the tested clinical strains, the average fluconazole MIC decreased from 244 μg/ ml to 38.46 μg/ ml after this prolonged pretreatment, and the average fluconazole MFC decreased from 254.67 μg/ ml to 66.62 μg/ ml but for the group of susceptible isolates, the fluconazole MIC decreased to an average of 0.52 μg/ ml, and the fluconazole MFC decreased to an average of 4.25 μg/ ml. Also terpinen-4-ol, the main bioactive component present in TTO, strongly enhanced fluconazole activity against fluconazole- resistant *C. albicans* strains; the terpinen-4-ol MICs for clinical *C. albicans* strains ranged from 0.06% to 0.25% (average= 0.11 ± 0.09%); exposure of fluconazole resistant clinical for 24h to fluconazole and sub-lethal doses (1/4 MIC) of terpinen-4-ol strongly enhanced fluconazole activity against these strains, and all of *C. albicans* isolates were classified as susceptible (fluconazole MIC decreased to 0.125 μg/ ml) (Mertas *et. al.*, 2015).

The anti-candidal activity of eugenol (main component of clove oil) and thymol (main component of thyme oil) alone or in combination was evaluated by investigating their ability to interfere with the architecture of the envelope of *C. albicans*. Both investigated strains were susceptible to thymol and eugenol at MIC values of 125 µg/ ml and 500 µg/ ml respectively. Almost all of the untreated *Candida* cells were round or oval in shape with smooth surfaces, but exposure to 1 MIC of eugenol or thymol alone induced a dramatic change in the morphology of the envelope. The number of normal round and smooth cells was significantly reduced and depending on the time of incubation there was a progressive increase in the number of damaged cells with rough and wrinkled surfaces, flattened cells with surface folds, cells with holes, collapsed cells and ghosts. Thymol proved to be about 40– 50% more active than eugenol. The same behaviour, but to a lesser extent, was observed when the *Candida* cells were incubated with 1/2 and 1/4 MICs of the two molecules alone, but incubation with 1/8 MIC of eugenol or thymol did not induce any significant alterations in comparison with controls. The combination of 1 MIC of eugenol plus 1 MIC of thymol induced a statistically significant increase in the number of damaged cells in comparison with the corresponding single concentrations of the two molecules. The effect of eugenol alone (+8.8± 1.48%), which could be seen after the first hour of incubation, was no different from that of the control (+7.80± 2.48%), and the corresponding effect of thymol was only 14± 4.18%, but the effect of the combination (31.4± 5.27% of damaged cells) was greater than the sum of the effects of the two single molecules alone. This behaviour, which was also observed after 2 h, had such a greater and unexpected candicidal effect after 4 h that the number of cells was reduced by more than ten times the corresponding values of the single molecules, and so it was impossible to find a representative number of cells to count. In any case, the residual cells were greatly damaged and there were hardly any normal cells. These findings indicate the presence of a synergistic effect when the two molecules are

combined, and the same was true after 4 h with the combination of 1/2 MIC of eugenol plus 1/2 MIC of thymol. The combinations of lower concentrations (1/4 MIC+ 1/4 MIC and 1/8 MIC+ 1/8 MIC) had progressively diminishing effects as expected but, once again a statistically significant increase in the number of damaged cells was observed after 2 h of incubation, whereas the concentrations of the single molecules alone had no effect. This is another finding indicating the presence of synergism when the two molecules were combined (Braga *et. al.*, 2007).

A study was conducted to evaluate the activity of *Ocimum basilicum* (Lamiaceae) leaves essential oil and its major components (linalool and geraniol) in enhancement of flucoconazole anti-fungal activity against the fluconazole sensitive and resistant strains of *Candida albicans* and *Cryptococcus neoformans*. The results showed that all combinations tested produced FIC index values ranging from 0.3826 to 0.6326. This showed that all these combinations reduced the MIC values. The synergistic effect was observed in the combination of flucoconazole and geraniol, reducing their MIC from 31.25 to 4.14 μg/ ml and 76 to 19 μg/ ml respectively, and in the combination of linalool with geraniol, reducing their MIC values from 790 to 111 μg/ ml and 76 to 19 μg/ ml respectively. When FLC was combined with 197 μg/ ml linalool and 625 μg/ ml EO, their MIC values were reduced from 31.25 to 8.054 μg/ ml and 4.14 μg/ ml in the respective combinations. It is important to note that, in combination, the needed concentrations of the two major components together to completely eradicate *C. neoformans* were very low. For the *C. albicans* strains all the combinations tested produced FIC index values ranging from 0.127 to 0.57. Although all the combinations reduced the MIC values in at least one of the paired substances, we did not observe any synergistic effect in the combinations of natural components with flucoconazole against *C. albicans* sensitive. However, a synergistic effect was observed in the combination of linalool with geraniol, reducing their MIC values from 790 to 105 μg/ ml and 152 to 38 μg/ ml, respectively. Furthermore, all combinations tested presented synergistic effect against *C. albicans* resistant. When flucoconazole was combined with essential oil 156 μg/ ml its MIC value was reduced from 500 to 1.01 μg/ ml. The combination of flucoconazole with 197 μg/ ml linalool and 38 μg/ ml geraniol reduced its MIC value from 500 to 2.02 μg/ ml and to 1.04 μg/ ml respectively. This is a significant result because when the high concentrations of the standard drugs were reduced the collateral effects were also reduced. Moreover, the combination of linalool and geraniol caused complete cellular inhibition at reduced MICs of 4.8 μg/ ml and 397 μg/ ml for geraniol and linalool respectively. These results corroborate with previously reported results which showed synergistic effect of geraniol and flucoconazole against *C. albicans*. However, when compared with this study, a higher concentration of geraniol (140 μg/ ml) was necessary to reduce the MIC value of flucoconazole from 64 to 2 μg/ ml (Cardoso *et. al.*, 2016).

Essential oils of the dried parts of *Origanum vulgare* (Lamiaceae), *Pelargonium graveolens* (Geraniaceae) and *Melaleuca alternifolia* (Myrtaceae) were tested with nystatin against some *Candida* species. The MIC and FIC values were determined by the micro dilution method. MIC of *O. vulgare* alone, MIC of one it with nystatin and its FIC ranged between 0.35- 0.7 mg/ ml, 0.04- 0.08 mg/ ml and 0.06- 0.12 mg/ ml respectively. The MIC of one single sample and MIC of one sample of the most effective combinations and FIC for nystatin ranges between 2 and 8 mg/ ml; 0.1 and 0.4 mg/ ml; 0.02 and 0.05 mg/ ml. The *P. graveolens* oil MIC of one single sample

and MIC of one sample of the most effective combinations and FIC ranges between 0.06 and 0.12 mg/ ml; 0.01 and 0.03 mg/ ml; 0.06 and 0.25 mg/ ml respectively. Few results were obtained to be additive (FICI = 40.5) for the associations nystatin with *M. alternifolia* essential oil. Also less effective results obtained with *P. graveolens* and few results additive effect (FICI = 40.5) for *M. alternifolia* essential oil. Also it has been shown that the nystatin essential oil combination administered against the *Candida* species is likely to reduce the minimum efficient dose of nystatin. In fact, as far as the strains under study are concerned, the concentrations used to assess the synergistic effect of the combinations were clearly lower than those of the MIC of each and any drug used. *O. vulgare* oil was the most effective among the oils that were tested in this study, for all strains considered for their synergistic effects, in all experiments carried out inhibiting all the *Candida* species evaluated in this study. Some combinations of nystatin and *P. graveolens* essential oil did not have any synergistic interactions for some of the strains considered. Associations of nystatin with *M. alternifolia* essential oil had only an additive effect (Rosato *et. al.*,2009).

The essential oils of two aerial parts Moroccan endemic thymes (*Thymus broussonetii* and *T. maroccanus*- Lamiaceae) with amphotericin B and fluconazole were tested against *Candida albicans*. Macro dilution broth method was used to determine MIC. Most of the essential oils tested singly exhibited significant inhibitory activity against *C. albicans* with MIC of around 0.25 mg/ ml. The FICIs calculated from the results of the checkerboard titer assays showed that combining amphotericin B or fluconazole with thyme oils from either species examined caused significant decrease in the MIC of the anti-fungal drugs when compared to their individual MIC values; for example, the MIC of amphotericin B alone against *C. albicans* was lowered from 16 to 4 g/ ml in the presence of *T. maroccanus* oil, the MIC of the *T. maroccanus* oil alone also was decreased from 0.25 to 0.0625 mg/ ml. Synergistic effects were obtained using various combinations of *T. maroccanus* and *Thymus broussonetii* essential oils with amphotericin B and fluconazole with FICI of 0.49, 0.27, 0.37 and 0.3 respectively. Also, the results indicate that the synergistic effect of essential oils with fluconazole was stronger than the combination with amphotericin B. All these data highlight that the essential oils tested potentiate the anti-fungal action of amphotericin B and fluconazole, suggesting a possible utilization of these essential oils in addition to anti-fungal drugs for the treatment of some candidiasis. The use of these combinations is likely to reduce the minimum effective dose of the drugs, thus minimizing their toxic side effects and the treatment cost (Saad *et. al.*, 2010).

The anti-fungal activity of the essential oil of aerial parts of *Agastache rugosa* (Lamiaceae) and its main constituent estragole were investigated alone and their combinations with ketoconazole against 10 fungi using broth micro dilution, disk diffusion and checkerboard micro-titre assays. The 10 tested fungi were *Aspergillus niger, A. flavus, Blastoschizomyces capitatus, Candida albicans, C. utilis, C. tropicalis, Cryptococcus neoformans, Trichoderma viride, Trichophyton tonsurans* and *Trichosporon mucoides*. The MICs of the essential oil of *A. rugosa* were generally lower than those of estragole for most of the tested fungi. This finding suggests that the activity of the oil fraction is based mostly on estragole component, which makes up half of the oil fraction, while the other constituents have relatively mild activity. Ketoconazole had much higher activity than either estragole or *A. rugosa* oil with MICs ranging between 12.5 and 25.0 µg/ ml. When ketoconazole was

combined with estragole it caused a remarkable decrease in the MICs compared with each compound alone in the tested fungi. An isobologram constructed with data from the checkerboard titre assay depicts the ketoconazole– estragole combination is synergistic. The essential oil of *A. rugosa* showed a similar synergistic effect with ketoconazole producing an FIC index of 0.19 in another checkerboard titre assay (Shin and Kang, 2003).

The anti-candidal effect of the essential oils from *Satureja montana* (Lamiaceae), *Lavandula angustifolia* (Lamiaceae), *L. hybrida* (Lamiaceae), *Syzygium aromaticum* (Myrtaeae), *Origanum vulgare* (Lamiaceae), *Rosmarinus officinalis* (Lamiaceae) and other chemotypes *of Thymus vulgaris* (Lamiaceae) on *Candida albicans* growth were studied individually. The strongest inhibitory effects were observed with the essential oils of *Thymus vulgaris* thymol chemotype (the effectiveness was mainly due to its content of thymol, 63.22%), and because of its promising activity it was studied in combination with amphotericin B against *C. albicans*; the results showed a very weak concentrations of essential oil produced a strong increase in the MIC 80%, the strongest increase being obtained with a concentration of essential oil equal to 0.0025 µg/ ml, then the MIC 80% decreased with lower concentrations of essential oil. It can be noted that for concentrations ranging from 0.01 and 0.3 µg/ ml a linear decrease of the MIC 80% of amphotericin B was observed, if this decrease of the MIC 80% can be attributed to the anti-fungal action of the essential oil, it is difficult to explain the increase of the MIC 80% (antagonistic effect) observed with lesser quantities of the oil. Though the very weak concentration of the oil exhibit strong antagonism, synergism was observed with when concentration increased (concentration dependent). The essential oil concentrations of 0.2 and 0.3 µl/ ml produced a decrease of the MIC 80% of amphotericin B compared with those of amphotericin B alone. The strongest decrease (48%) was obtained with a medium containing 0.2 µl/ ml of essential oil; an essential oil concentration of 0.3 µl/ ml produced a total inhibition of the fungal growth with a MIC 80% of amphotericin B equal to zero, therefore the presence of amphotericin B in the culture medium was not necessary. This study supports the potential role of essential oils from *Thymus vulgaris* thymol chemotype as an anti-fungal agent. The potentiation of amphotericin B exhibited by this essential oil may be promising for more effective and less toxic therapy for the treatment of mycoses (Giordani *et. al.*, 2004).

The anti-fungal activity of the essential oil from *Cinnamomum cassia* (Lauraceae), alone and combined with amphotericin B were investigated. The composition of the oil was analysed by GC/MS and characterized by its very high content of cinnamaldehyde (92.2%). The minimal inhibitory concentration (MIC 80%) used to evaluate the anti-fungal activity against *Candida albicans* was determined by a macro broth dilution method. The results showed an increase of MIC with essential oil concentrations ranging from 0.08 to 0.5 µl/ ml and a decrease of MIC 80% was observed by comparison with that of amphotericin B alone; the strongest decrease (70%) was obtained with a concentration of 0.1 µl/ ml, this decrease can be attributed to the anti-fungal effect of the essential oil. This potentiation of amphotericin B obtained *in vitro* may show promise for the development of less toxic and more effective therapies especially for the treatment of candidal infections associated with HIV infection (Giordani *et. al.*, 2006).

Essential oils of *Cymbopogon martini* (Poaceae) and *Chenopodium ambrosioides* (Amaranthaceae) leaves were tested for their anti-fungal activity, the oils were tested singly and in combination against dermatophytes and some filamentous fungi *in vitro* as well as *in vivo* by applying an ointment on a guinea pig model. The MIC of the essential oils (either individually and their combination) were compared for its effectiveness with the MIC of commonly used synthetic drugs (i.e. griseofulvin, ketoconazole and fluconazole). In *in vitro* study, both the essential oils, alone and their combination, exhibited significant inhibitory activities against the tested dermatophytes; the MICs of *C. martini* against *Microsporum gypseum* and *Trichophyton rubrum* were 200 and 150 ppm respectively, and are comparatively less than the MICs of *C. ambrosioides* against *M. gypseum* (700 ppm) and *T. rubrum* (350 ppm). The MICs of combination of oils were also less than that of *C. ambrosioides* against *M. gypseum* (500 ppm) and *T. rubrum* (250 ppm). MLC values of essential oils and their combination ranged from 500 to >1,000 ppm against dermatophytes. *T. rubrum* was found to be more sensitive than *M. gypseum* against essential oils. On the other hand, the MIC values of griseofulvin, ketoconazole and fluconazole ranged from 1,000 to 5,500 ppm, which are much greater than the MICs of essential oils and their combination (150–700 ppm). In *in vivo* study, essential oil ointments were prepared and applied against induced ringworm in guinea pig model and disease removal was observed in 7– 21 days, at day 5 of the treatment, randomly selected hairs of the inoculation areas were found to be positive for fungal culture on Sabouraud Dextrose Agar (SDA). All the essential oil ointments were recorded to be efficacious (*C. martini* > oil combination > *C. ambrosioides*) in a time-dependent manner. *C. martini* resulted in the complete cure of *T. rubrum* and *M. gypseum* infection at day 17 and day 21 respectively, while *C. ambrosioides* and oil combination cured the disease in most of the treatment models at day 21. Both the essential oils and their combination displayed strong anti-fungal effects. The results provide a scientific validation for the use of these essential oils in the treatment of dermatophyte infections and may be recommended as an alternative to synthetic drug for topical application because of their activity and synergism (Prasad *et. al.*, 2010).

Antifungal activity of *Coriandrum sativum* (Apiaceae) essential oil alone and it's in combination with amphotericin B was studied against two strains of *Candida albicans* and one strain of *C. tropicalis* using a micro dilution broth susceptibility assay and Checkerboard assay. The individual results showed coriander essential oil has a fungicidal activity against the *Candida* strains tested with MLC values equal to the MIC value and ranging from 0.05 to 0.4% (v/v). The evaluation indicates that the fungicidal effect was a result of cytoplasmic membrane damage and subsequent leakage of intracellular components such as DNA. Also, concentrations bellow the MIC value caused a marked reduction in the percentage of germ tube formation for *C. albicans* strains. A synergetic effect between coriander oil and amphotericin B was also obtained for *C. albicans* strains, while for *C. tropicalis* strain only an additive effect was observed. This study could be useful in designing new formulations for candidosis treatment (Silva *et. al.*, 2011).

The activities of essential oils from *Allium sativum* for. *pekinense, A. cepa* and *A. fistulosum* (Liliaceae) against three *Trichophyton* species (i.e. *T. rubrum, T. erinacei* and *T. soudanense*) responsible for severe mycoses in humans were investigated and compared with the activity of allicin. The fungistatic activities of *Allium* oils, allicin

and ketoconazole among others were singly evaluated by broth dilution method and disk diffusion assay. From the results, *A. sativum* for. *pekinense* oil was the most potent inhibitor of all three *Trichophyton* spp, with MICs of 64 mg/ ml, equivalent to 25– 50% of the activity of allicin (16– 32 mg/ ml). The combined effect of either *A. sativum* or allicin with ketoconazole were tested by the checker board titer test. The startling combination effects of ketoconazole and *A. sativum* oil were seen in FICIs ranging from 0.09 to 0.12 against *Trichophyton* spp. It showed significant synergism of ketoconazole in combination with *A. sativum* volatile oil fraction, and also with allicin. Moreover, the greater than four-fold difference in width of the inhibited zone between *A. sativum* volatile oil fraction and allicin following separate administration was increased significantly by combination of these samples with ketoconazole. Ketoconazole combined with allicin resulted in additive effects, with FICIs from 0.53 to 0.75 (Pyun and Shin, 2006).

The growth fungal inhibition of six herbal essential oils were tested against three *Trichophyton* spp (*T. schoenleinii, T. erinacei* and *T. soudanense*) alone and in combination with flucoconazole. Among the oil fractions tested alone, *Cymbopogon citratus* leaf (Poaceae) and *Eucalyptus globulus* leaf (Myrtaceae) were the most potent inhibitors of all tested fungi, with MIC of < 0.125– 0.25 mg/ ml and MFC of < 0.125– 1 mg/ ml. As expected, *Thymus vulgaris* (Lamiaceae) and its main component thymol had an especially high MIC value ranging between 0.25 and 1 mg/ ml, thymol was more active than the total oil fraction of *T. vulgaris*. The oil of *Pelargonium graveolens* (Geraniaceae) as well as its main components (citronellol and geraniol) strongly inhibited these fungi, with MIC of 0.25– 2 mg/ ml, and because of its strong effect, the combined effects between *P. graveolens* oil and its main components (citronellol and geraniol) with fluconazole were evaluated using a checkerboard microtitre assay against *Trichophyton* spp. The MIC of ketoconazole combined with *P. graveolens* oil were remarkably decreased, with FIC indices ranging between 0.18 and 0.56. Moreover, fungal susceptibility to ketoconazole was enormously improved by combination with the oil or its main components. In an experiment with *T. erinacei* and *T. soudanense*, the FIC of ketoconazole when combined with citronellol or geraniol was 0.06 and 0.13 respectively. FIC indices indicate the strongest synergism between *P. graveolens* oil and ketoconazole against *T. soundanense*, with an FIC index of 0.18. Similar results were obtained by the combination of ketoconazole with geraniol or citronellol, with FIC index of 0.18 (Shin and Lim, 2004).

Essential oils from *S*tems and leaves of 56 colombian plants [including *Thymus vulgaris* (Lamiaceae), *Zingiber officinae* (Zingiberaceae), *Cunila origanoides* (Lamiaceae), *Eucalyptus citriodora* (Myrtaceae), *Morinda royoc* (Rubiaceae), *Lippia origanoides* (Verbenaceae) and *Piper bredemeyeri* (Piperaceae)] were assayed for anti-fungal activities alone and in combination with itraconazole and amphotrericin B against *Candidas albicans* clinical isolates. The most active samples were the oils from *P. bredemeyeri* (MIC range 157.5- 222.7 µg/ ml) and *L. origanoides* (MIC range 157.5- 198.4 µg/ ml). Also *M. royoc* was active (MIC= 250 µg/ ml). A most synergistic effect was obtained for the combination of itraconazole and *P. bredemeyeri* (FICI range 0.09- 0.13), but no interaction was detected for the combination of *P. bredemeyeri* and amphotericin B (FICI= 1.06) (Tangarife-Castaño *et. al.*, 2011).

The aerial parts of ten medicinal plants [including *Salvia officinalis* (Lamiaceae), *Pelargonium graveolens* (Geraniaceae), *Eucalyptus globules* (Myrtaceae), *Pistacia lentiscus* (Anacardiaceae), *Thymus capitatus* (Lamiaceae), *Nigella sativa* (Ranunculaceae) seeds, *Cinnamomun verum* (Lauraceae) barks and *Syzygium aromaticum* (Myrtaceae) clove buds], among others were collected at the flowering stage and extraction of their essential oils was performed by hydro-distillation. The essential oils were investigated for anti-candidal activity and were evaluated for their potential synergism with fluconazole. Only *C. verum, T. capitatus, S. aromaticum* and *P. graveolens* exhibited a broad spectrum of activity against a variety of pathogenic *Candida* strains. Synergistic effect was observed with the combinations of *C. verum*/ fluconazole and *P. graveolens*/ fluconazole with FIC value 0.37. Investigation of the mechanism of action revealed that *C. verum* essential oil reduced the quantity of ergosterol to 83%, while *P. graveolens* essential oil may disturb the permeability barrier of the fungal cell wall, furthermore, the combination with fluconazole may affect ergosterol biosynthesis and disturb fatty acid homeostasis in *C. albicans* cells as the quantity of ergosterol and oleic acid was reduced to 52.33 and 72% respectively. Naturally occurring phytochemicals in *C. verum* and *P.graveolens* could be effective candidate to enhance the efficacy of fluconazole-based therapy of *C. albicans* infections (Essid *et. al*., 2017).

Isolated phytochemical(s) – drug(s) combinations:

In a study to evaluate the improved efficacy of anti-fungal drugs in combination with monoterpene phenols, the anti-fungal activity of phenolic compounds (i.e. carvacrol, thymol, eugenol and methyl eugenol) with anti-fungal drugs (i.e. fluconazole, amphotericin B, nystatin and caspofungin) were tested against 25 clinical isolates of *Candida auris.* MIC results showed that all compounds have anti-fungal activity at varying levels, it was evident that carvacrol had the best MIC values (125 µg/ ml), followed by thymol (MIC of 312 µg/ ml). It can also be seen that MFC values for all the four compounds were 1–2 folds higher than their respective MIC values. Carvacrol as it was the most active compound, its combination with fluconazole, amphotericin B, nystatin and caspofungin resulted in synergistic and additive effects in 68%, 64%, 96% and 28% respectively, the Combinations also reduced the MIC values of all drugs. Therefore it has potential to be developed into a novel anti-fungal agent (Shaban *et. al*., 2020).

A research was conducted to determine the anti-fungal activity of curcumin obtained from *Curcuma longa* (Zingiberaceae) and to explore the possibilities of its use in a combination with fluconazole and itraconazole. The MIC of fluconazole, itraconazole and curcumin was found to be in range of 32- 64 µg/ ml, 8- 32µg/ ml and 64- 256 µg/ ml respectively. Further, the results of the *in vitro* anti-fungal study was based on the comparative zone of inhibition measurements of the prepared combinations at a concentration of 10 µg/ ml. The results indicate that the presence of curcumin significantly increases the anti-fungal capacity of both fluconazole and itraconazole. The FIC index was measured, and showed the increase of anti-fungal activity may be due to synergistic or additive effects. Furthermore, the topical sensitivity of the optimized combinations was determined by using rabbit vaginal model and were found to be free from any major sign of sensitivity (Choudhury et. al., 2019).

Zwiebelane A, a Cyclic Organo-sulfur Compound from Onion (*Allium cepa*; Liliaceae) was tasted alone and in combination with Polymyxin B to evaluate its activities in fungal vacuole disruption against *Saccharomyces cerevisiae.* Zwiebelane A itself is ineffective in inhibiting the growth of *S. cerevisiae* cells at 1.2 mM, whereas polymyxin B shows a static growth-inhibitory effect at 60 µg/ ml. The yeast cells were subjected to lethal damage when polymyxin B was added in combination with zwiebelane A. The normal architecture of the vacuoles was maintained when cells were treated with either polymyxin B alone at 60 µg/ ml or zwiebelane A alone at 1.2 mM, but the vacuoles were clearly disrupted as a result of the combined actions of polymyxin B and zwiebelane A (Borjihan *et. al.,* 2010).

Three saponins (ceposide A, ceposide B, and ceposide C) were isolated and extracted by acetone from the bulbs of white onion, *Allium cepa* (Liliaceae), they were evaluated for their antimicrobial activity alone and in combinations against ten fungal species, i.e. three soil-borne pathogens (*Fusarium oxysporum* f. sp. *lycopersici*, *Rhizoctonia solani and Sclerotium cepivorum*), five air-borne pathogens (*Alternaria alternata, Aspergillus niger, Botrytis cinerea, Mucor* spp and *Phomopsis* spp) and two antagonistic fungi (*Trichoderma atroviride* and *T. harzianum*) were selected. The results indicated that anti-fungal activity of all three saponins increased with their concentration and varied with the following rank: ceposide B > ceposide A > ceposide C. In the bioassays where saponins were applied in combination, additive effects were generally observed. However, it was found a significant synergism (when applied in even mixture of 33.3% for each ceposide compound) against *B. cinerea* and *T. atroviride*, growth of these two fungi was strongly inhibited when saponins were applied in combination with *B. cinerea* showing a much larger inhibition at 10 and 50 ppm (Lanzotti *et. al*., 2012).

Synergistic effects of tea catechin, epigallocatechin gallate (EGCG), alone and in combination with some common anti-mycotics against oral *Candida* spp was evaluated. The MIC of EGCG, miconazole, fluconazole and amphotericin B against biofilms of *C. albicans, C. parapsilosis, C. tropicalis, C. glabrata, C. kefyr* and *C. krusei* were determined by micro-dilution method. The results showed that EGCG inhibited the growth of the tested *Candida* spp at concentrations ranging from 375 to 1,500 mg/ ml. However, it showed synergistic anti-fungal effects against most *Candida* spp when combined with miconazole, fluconazole and amphotericin B with FICI ranging from 0.15 to 0.50. When EGCG was in combination with miconazole and amphotericin B, synergistic effects were observed against all tested species (MICs of miconazole reduced from 0.25– 1 to 0.031– 0.25 mg/ ml; MICs of amphotericin B reduced from 0.063– 0.25 to 0.016– 0.063 mg/ ml). For EGCG and fluconazole combination, however, no synergism was observed against *C. glabrata, C. krusei* and *C. kefyr*. (Ning *et. al*., 2015).

Acteoside from aerial parts of *Colebrookea oppositifolia* (Lamiaceae) were extracted by 50% aqueous ethanol. Acteoside and amphotericin B were *in vitro* tested each one alone and in combination against clinical isolates of *Candida albicans, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis, Cryptococcus neoformans, Aspergillus flavus, A. fumigatus, A. niger* and *A. parasiticus* alongside with one reference strain. The MIC of amphotericin B was measured in the absence and presence of increasing concentrations of acteoside (0.195–12.5 mg/ml) using a two-dimensional checker

board micro broth dilution method. Acteoside on its own did not show any anti-fungal activity when tested at concentrations up to 1,000 mg/ml. However, interestingly, it showed a potent synergism in combination with amphotericin B against all tested fungal species, with FIC indices in the range of 0.0312–0.1562. The FIC of amphotericin B when combined with acteoside ranged from 0.0156 to 0.125 for yeasts (8–64 fold reduction in amphotericin B MIC) and was 0.125 for *Aspergillus* spp (8-fold reduction in amphotericin B MIC). A more prominent synergistic interaction between amphotericin B and acteoside was observed in *Cryptococcus neoformans* (FIC index 0.0312: 64-fold reduction in amphotericin B MIC), among all tested fungal species. Again the fungicidal effect of the combination of amphotericin B with acteoside was assessed on *C. albicans*, *A. fumigatus* and *C. neoformans*. Amphotericin B was used at a concentration of 0.256 x MIC (0.1256 x MIC for *C. neoformans*), as well as in combination with increasing concentrations of acteoside ranging from 0.78 to 12.5 mg/ ml. As expected, amphotericin B alone at these concentrations did not show any inhibitory activity, whilst the fungicidal activity (99.9% kill) was achieved at 46 x MIC (26 x MIC for *C. neoformans*) in 24 h when compared with the growth of control. However, the same sub-inhibitory concentrations of amphotericin B resulted in fungicidal activity when tested in combination with acteoside at concentrations of 0.78–12.5 mg/ ml for 24 h. It's to say the fungicidal activity of the combination was equivalent to the fungicidal activity of amphotericin B alone at 46 x MIC (26 x MIC for *C. neoformans*) because of the synergistic interaction (Ali *et. al*., 2011).

In vitro anti-fungal activity of naturally occurring phenyl propanoids, eugenol and methyleugenol alone and in combination against 64 fluoconazole-sensitive and 34 fluoconazole-resistant clinical *Candida* isolates was highlighted. The nature of the interaction was studied from FICIs for both eugenol plus fluoconazole and methyleugenol plus fluoconazole combinations calculated from checkerboard micro dilution assays. The MICs of eugenol and methyleugenol against *C. albicans*, *C. tropicalis* and *C. glabrata* ranged from 475 to 500 mg/ ml and 340 to 350 mg/ ml respectively. The MIC of fluoconazole was 2.5– 7.5 mg/ ml and was within the reference ranges. It was noted that isolates intrinsically resistant to fluoconazole (MICs 80– 110 mg/ ml) also showed sensitivity to both compounds eugenol and methyleugenol. However, it is clear that methyleugenol proved to be more active against all *Candida* strains than eugenol. The anti-fungal activity of both compounds increased with increasing their concentration. All the fluoconazole-susceptible and resistant *Candida* isolates showed high degree of sensitivity as was evident from large inhibition zones. The results of the checkerboard micro-titre assay indicated significant combined effects between eugenol/ methyleugenol and flucoconazole for flucoconazole-sensitive and flucoconazole-resistant *Candida* isolates. FICI values for eugenol plus fluoconazole and methyleugenol plus flucoconazole combinations against all flucoconazole-sensitive *Candida* isolates studied ranged from 0.31 to 0.55 and 0.24 to 0.58 respectively. Out of 64 flucoconazole-susceptible *Candida* isolates tested, the interaction between eugenol and flucoconazole was synergistic in 58, whereas for methyleugenol and flucoconazole, 59 showed synergy and only 5 isolates showed indifference by the FICI method. Out of 34 flucoconazole-resistant *Candida* strains tested, 29 and 31 isolates showed synergistic affects for eugenol plus flucoconazole and methyleugenol plus flucoconazole respectively. No antagonistic activity was seen in the strains tested in the present study. From these results we

suggest that eugenol and methyleugenol have great potential as anti-fungals, and that fluoconazole can be supplemented with eugenol and methyleugenol to treat flucoconazole-resistant Candidal infections (Ahmad *et. al.*, 2010).

Evaluation of the *in vitro* activity of baicalein, the flavone constituent of *Scutellaria baicalensis* (Lamiaceae), and its combination with fluconazole against *Candida albicans, C. tropicalis* and *C. parapsilosis* was conducted. The MIC_{50} of baicalein alone ranged from 13 to 104 mg/ ml. In this study, exposure to baicalein at MIC_{50} values obtained for each strain and at 260 mg/ ml resulted in high loss of viability, revealed by a reduction in fungal colony counts compared with the untreated control, suggesting that baicalein is capable of anti-candidal activity. The anti-fungal activity of fluconazole plus baicalein was greater than the individual contribution of each agent, according to FICI values, the combination demonstrated partial synergistic properties against *C. albicans* and *C. tropicalis*; the combination of baicalein and fluconazole produced a synergistic action against *C. parapsilosis*, in this case, the fluconazole MIC changed from susceptible dose-dependent (MIC_{50}= 516 mg/ ml) to susceptible (MIC_{50}= 50.125 mg/ ml), for *C. parapsilosis*, baicalein significantly potentiated the anti-candidal effect of fluconazole. Thus, the combination of baicalein with fluconazole may represent an attractive prospect for the development of new management strategies for candidiasis caused by *C. parapsilosis* (Serpa *et. al.*, 2012).

The activity of catechins isolated from green tea leaves of Assam *Camellia sinensis* (Theaceae) were tested alone and in combinations with fluconazole, amphotericin B and copper sulphate against some *Candida* spp following micro-dilution checkerboard technique and time kill assay. The MIC_{90} of purified catechins against *C. albicans* was observed at 125 mg/ ml while minimum fungicidal activity (MFC) was depicted between 250 mg/ ml to 1 mg/ ml. The MIC of Fluconazole was depicted at concentration of 64 mg/ ml and 128 mg/ ml for *C. albicans* and *C. glabrata* respectively, while MIC of Amphotericin B was observed at concentration of 1 mg/ ml against both *C. albicans* and *C. glabrata*. Purified catechins of showed synergistic activity with fluconazole and amphotericin B against *Candida* spp. Time kill assay depicted synergistic activity at minimum inhibitory concentration and twice of minimum inhibitory concentration of purified catechins and its combinations. Further, copper sulphate increased the anti-candidal efficacy of synergistic combinations by 0.4% to 6.63%. It can be inferred that present evaluated synergistic composition can confer promising anti-candidal efficacy and requires further investigation of safety and translational guidelines for effective and safer green tea based potent therapeutic drug (Anand and Rai, 2017).

The *in vitro* anti-fungal effects of osthole, a natural coumarin compound derived from *Cnidium* plant (Apiaceae), was investigated alone and in combination with fluconazole against Fluconazole-resistant *Candida albicans.* A total of 30 clinical fluconazole-resistant *C. albicans* isolates (MIC_{50} greater than or equal to 8 µg/ ml) with unknown drug resistance mechanisms, and 10 fluconazole-susceptible C. *albicans* isolates (MIC_{50} less than or equal 1 µg/ ml) were used in this study. The results showed that osthole did not exhibit any anti-fungal effect when it was used alone, with an MIC_{50} of greater than 64 µg/ ml. Compared to osthole alone, its combination with fluconazole showed a significant synergistic effect against the fluconazole-resistant *C. albicans* by reducing the dose of fluconazole 1 to 16 µg/ ml,

and the dose of osthole 4 to 16 µg/ ml, also the FICI 0.04 to 0.31. Further, a growth curve assay confirmed the synergism of fluconazole and osthole against fluconazole resistant *C. albicans* and indicated that the synergistic effect was in a dose-dependent manner with osthole. Unlike the results of fluconazole-resistant isolates, fluconazole and osthole did not exhibit synergism on fluconazole-susceptible strains, since the FICI was 0.51 to 2.01 (Li *et. al.*, 2017).

Anti-fungal effects of cinnamaldehyde, eugenol, honokiol, shikonin, magnolol and shikonin were evaluated alone and in combination with fluconazole against *Candida spp*. When the phytocompounds tested alone, some displayed potent anti-fungal activity, with MICs of ≤ 8µg/ ml, and several compounds were more effective than fluconazole or itraconazole (e.g. honokiol, magnolol and shikonin). In the group of phenylpropanoids, some phytocompounds were demonstrated to have slight or moderate efficacy, such as cinnamaldehyde, eugenol and magnolol. However, in combination with fluconazole they showed significant synergistic effects, including resistant strains and biofilms of *Candida spp*. For instance, when eugenol, methyleugenol and magnolol were used in combination with fluconazole against some strains of *Candida* isolates, the FICI values depicted a high synergism (FICI<0.5) of fluconazole with all compounds. When terpenoids interacting with *Candida spp*. as a toxic agent, farnesol displayed synergistic or additive interactions with fluconazole against drug-resistant *Candida* isolates or *C. albicans* biofilms. (Lu *et. al.*, 2017).

Anti-candidal activity of two asarones (α and β) purified from *Acorus calamus* (Acoraceae) extracted with alcohol were tested, in combination with three clinically used anti-fungal drugs (fluconazole, clotrimazole and amphotericin B). The tested organisms were *Candida albicans* and *C. tropicalis*. The MIC values showed that highest activity was recorded for β-asarone in the range of 64– 125 µg/ ml, while α-asarone exhibited the activity at higher concentration (250– 500 µg/ ml), for azole drugs the activity ranged from 1 to 4 µg/ ml and for amphotericin B the activity ranged from 1 to 2 µg/ ml. The combined anti-candidal activities of asarones and the chosen drugs were assessed using the checkerboard micro-dilution and time-kill assays, the results showed significant synergistic interaction. Significant synergistic interaction was recorded by β-asarone with azoles and amphotericin B. Antagonism and indifference was not recorded for any combinations. In the combination of α and β asarones with azoles and amphotericin B, the MIC values have reduced to more than eight times (Kumar *et. al.*, 2015).

The seeds of *Peganum harmala* (Nitrariaceae) contain about 2 to 6% pharmacologically active alkaloids which are mostly β carbolines such as harman, harmine, harmaline and harmalol. The β-carboline alkaloids were extracted by methanol through a bioassay-guided fractionation and their anti-fungal activities were investigated alone and in combination against *Aspergillus niger* and *Candida albicans*. The isolated β-carboline alkaloids showed anti-microbial effects against all tested microorganisms. Diameters of inhibition zones ranged between 10.5 and 31.5 mm. When the alkaloids were examined individually, *C. albicans* was the most susceptible to harmine with inhibition zones of 22.2 mm, while harman was the most active against *A. niger* with inhibition zones reaching 20.8 mm. Harmaline was more effective against *C. albicans* (the diameter of inhibition zone was 21.3 mm), meanwhile, harmalol showed moderate activities. A combination of harman and

harmaline mixture was effective against *C. albicans* (the inhibition zone was 29 mm). The lowest minimal value of 0.333 mg/ ml was recorded with the total (crude) harmala alkaloids and the mixtures of harman with harmine or harmaline (Nenaah, 2010).

The *in vitro* activities of carvacrol, cinnamaldehyde and thymol, alone and in combination with fluconazole, itraconazole, ketoconazole, clotrimazole, miconazole, terbinafine and nystatin against *Malassezia pachydermatis* were investigated. The combination results showed the presence of synergism, indifference and antagonism, the highest synergistic interaction (80%) based on the MIC values was observed for the following combinations: thymol + nystatin, carvacrol + nystatin and carvacrol + miconazole, the other combinations produced synergistic interactions that ranged from 16.6% to 70%. The highest percentage of indifference (70%) was observed for cinnamaldehyde + fluconazole, thymol + terbinafine and cinnamaldehyde + terbinafine. The highest antagonistic effects were detected from the combinations of carvacrol + ketoconazole, thymol + ketoconazole (40%) and cinnamaldehyde + ketoconazole (46.6%) (Schlemmer *et. al.*, 2019).

The *in vitro* interactions of flavonoids with fluconazole against *Candida tropicalis* strains resistant to fluconazole were investigated alongside the mechanism of synergism. Three combinations formed by (+) hydrated catechin, hydrated quercetin and (-) epigallocatechin gallate at a fixed concentration with fluconazole were tested. All strains studied showed MIC_{50} value of 64 µg/ ml for flucoconazole. The flavonoids alone had no anti-fungal activity within the concentration range tested, but when they were used as a co-treatment with fluconazole, there was significant synergistic activity. The synergism between the flavonoids and fluconazole was determined using the checkerboard technique, in which the association of flavonoids with fluconazole showed a synergistic effect on fluconazole-resistant strains and exhibited FICIs ranging from 0.25 to 0.38 µg/ ml (a synergistic effect is FICI of < 0.5) (da Silva *et. al.*, 2014).

Plant latex – drug(s) combinations:

Euphorbia characias (Euphorbiaceae) latex was collected from experimental plantations by making repeated cuts along the stems, and was collected in Eppendorff tubes and stored at 4 °C until required. The *in vitro* suseptibility of *Candida albicans* to ketoconazole and *E. characias* latex alone or in combination was tested using the macro-broth dilution method. The concentration of latex was estimated by its proteins contents by Bradford's method. The MIC 80% of the crude latex and ketoconazole were 159 µg protein/ ml and 0.3901 µg/ ml respectively. The utilization of the mixture of latex at several concentrations (7.8, 15.62, 31.25, 62.5 and 125 µg protein/ ml) and ketoconazole indicates a synergistic effect between the latex and ketoconazole; for latex concentrations of 31.25 and 62.5 µg protein/ ml the MIC 80% of ketoconazole were inferior (0.194 and 0.183 µg/ ml respectively) to that obtained with ketoconazole alone (0.390 µg/ ml) (Giordani *et. al.*, 2001).

Carica papaya (Caricaceae) latex sap (0.41 mg protein/ ml) in combination with fluconazole (2 µg/ ml) were tested against C*andida albicans* growth. The mixture showed synergistic action, this synergistic effect resulted in partial cell wall degradation as indicated by transmission electron microscopy observations. An

increase of fluconazole concentration from 2 µg/ ml to 4 µg/ ml involved in a small decrease of MIC80% from latex (150 to 130 µg protein/ ml) (Giordani *et. al*.,1997).

The *Hevea brasiliensis* (Euphorbiaceae) latex obtained from the rubber trees was ammoniated (5% at final concentration) to prevent rubber coagulation, amphotericin B was dissolved in 100% DMSO. The anti-fungal activity of the latex was observed with various fungi strains in macro-broth dilution assays and the MIC80% was determined singly and in combination with amphotericin B. In individual assay, it was concluded that the growth of all tested yeasts were inhibited with *H. brasiliensis*, the strongest antifungal effect was obtained with *Trichosporon cutaneum* (MIC80% = 40.615 µg protein/ ml) and *Cryptococcus neoformans* (MIC80% = 56.078 µg protein/ ml). In the assay of combination, *Candida albicans* cultured on medium supplemented with a mixture of *H. brasiliensis* latex and amphotericin B, the MIC80% of amphotericin B were measured with *C. albicans* cultured in presence of several constant concentrations of the latex ranging from 7.5 to 60 µg protein/ ml, the best MIC80% (0.201 µg amphotericin B/ ml) was measured when culture medium contained 60 µg protein/ ml, the use of 15 and 30 µg protein/ ml gave MICs that were slightly higher (0.221 and 0.247 µg protein/ ml respectively), a stronger MIC80% (0.369 µg amphotericin B) was obtained with utilization of 7.5 µg protein/ ml in culture medium. According to the results, MIC80% decreases strongly for the latex concentrations in a range 0– 15 µg protein/ ml, then decreases very slightly with higher concentrations up to 60 µg protein/ ml. In conclusion, amphotericin B was synergized with all *H. brasiliensis* latex concentrations tested, the rates of synergy were about 50, 44 and 55% with 15, 30 and 60 µg protein/ ml latex respectively (Giordani *et. al.*, 2002).

Conclusion:

As proved by this review, the number of plants extracts acting in synergy with each other, or with known antifungal drugs, against different fungal strains is too large. Our investigations concluded that the combination of medicinal plants extracts (including essential oils, isolated phytochemicals and plant latexes) with known antifungals of known activities offers significant potential for the development of novel antifungal therapies that can treat fungal infections caused by many resistant fungal organisms. Since some of combinations as showed from this review can lead to either hamful or antagonistic effect, there is an urgent need for more studies (both *in vitro* and *in vivo*) concerning plant(s) – plant(s)/drug(s) interactions to understand the synergistic mechanism of action, which is fundamental in the development of new pharmacological agents to treat fungal infections effectively and safely.

aNOTE:

The study highlights the efficacy of "herbal medicine" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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