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, 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 mentioned reasons many people, especially in developing countries, use medicinal plants (either alone, combined together or combined with known antifungal drugs) 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 in 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, so our review highlights many interactions between medicinal plants constituents with current available antifungal agents, these interactions may be synergistic, additive, indifferent or antagonistic, so, if there is any antagonistic effect, we recommend to avoid using the combination which caused this effect. 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 safely and effective drugs, specially with those combinations which showed synergistic effect.

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

Introduction:

The relationship between man and plants started from the earliest history and was developed, so, the herbalist is one of the first professionals in the evolution of human cultures. Today, with the improvements in technology and isolation methods, the plant kingdom is regarded as a big warehouse which contains many chemical compounds, some provide novel structures (lead compounds) from which synthetic chemists may derive even more interesting compounds (El Ghazali *et. al.*, 2003).

Over the last few decades, there has been an universal considerable interest in traditional and complementary medicine (TCM). The World Health Organization (WHO) stated the main role of herbal medicines in preventive, promotive and curative on healthcare system, especially in developing countries (WHO, 2013).

A survey done in 2007 demonstrated that about 15% of patients are taking herbal products in conjunction with conventional pharmacotherapy, among these patients, herbal-drug interactions were observed in about 40% of patients; but it is often difficult to establish exactly what is the causative agent of this herbal-drug interaction, especially if it occurs in patients receiving multiple drug therapies (polypharmacy). This is a substantial public health issue since many patients who are known to be using one or more prescription drugs are also taking supplements preparations, and unfortunately only about one third of these patients were reported to tell their physicians about their use of these products together (Gül Dülger, 2012).

“Generally, there is a wrong belief concept in the public population 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).

“If two or more phytochemical compounds and/or plant extracts were combined together, and this combinations lead to changes in the overall biological effects and/or the bioavailability of each component, this will result in herbal-herbal or herbal-drug interactions. These interactions may be synergy if the mixtures enhance (for example) the antioxidant status, anti-inflammation, anti-cancer and chemoprevention of several oxidative stress” (Phan *et. al.*, 2018). “In some cases, however, the combination of these mixtures may cause lower biological effects if they are administered in inappropriate ratios” (Iwuchukwu *et. al.*, 2011). “Sometimes the interactions of these mixtures may enhance or reduce the bioavailability of active compounds, often depending on many factors, like facilitation or competition in biological receptors or cellular uptakes and transportations” (Reboul *et. al.*, 2007). “On other hand, different classification methods were used to describe these positive or negative mixture interactions, they are potentiation, addition, synergy, or antagonism. There is a confusion about the differences between potentiation and synergy. If one active mixture(s) was/were mixed with another inactive mixture(s), but both mixtures produced a greater effect than that of the active mixture(s), the effect is known as potentiation (i.e. the presence of inactive mixture(s) enhanced the potency of active mixture(s))” (Efferth and Koch, 2011). If both mixtures were individually active, they

can produce an additive, synergistic or antagonistic effect if they were combined together" (Chou, 2006). Synergy comes from the Greek word "synergos", which means "working together". According to McGraw–Hill Concise Dictionary of Modern Medicine, synergy or synergism is defined 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). "Broadly, synergy is 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). "It is well known that herbal or plant extracts consist of a complex mixtures of natural compounds, this complexity may serve as a valuable resource for network-based multi-target drug discovery due to its potential treatment effects by synergy, for example, polyphenols and terpenoids are two groups of natural compounds which are available in many extracts, the former possess a strong binding ability to different molecular structures like proteins or glycoproteins, while the latter have a great affinities for cell membranes and therefore, a high potential to permeate through cell walls of the body" (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). Additive interaction is used to describe an effect of two combined active mixtures which produced similar potency of individual active components in each mixture; while antagonistic interaction appears when the combined effect is less potency than the sum of individual components potency of the mixture (Zhang *et. al.*,2019).

"Some fungi organisms have a harmful influence on the health and livelihood of mankind. The diseases caused by fungi are termed mycoses, today fungal infections are among the most difficult diseases to manage" (Köhler *et. al.*, 2017). "Figures say that there are about 1.5–5 million fungal species on the planet, only several hundreds of them can harm and 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's effector cells. In addition to the high increase in fungal infections caused by opportunistic and pathogenic fungi in compromised patients (like HIV positive patients), which are mainly 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 fungal infections by 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).

Hence fungal infection diseases are 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 which are limited to the outermost layers of the skin, nails, hair and the mucous membranes.

(ii) The subcutaneous mycoses: These are infections which are involved in the dermis, subcutaneous tissues and adjacent bone.

(iii) The systematic mycoses: These are infections that usually originate in the lung, but may spread to many other organs by blood or lymph circulation (Malcolm D. Richardson and David W. Warnock, 2012).

In comparison with the clinical availability of antibacterial drugs, there are very few number of antifungal agents. There are four major families of antifungal compounds: the polyenes, the azoles, the allylamines and the echinocandins. In addition, there is a miscellaneous group of antifungal 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 following table **(Table 1)** shows some studies which had tested a combination of two or more plant extracts together as antifungal agents, and showed if there is any synergisim, indifferent, additive or antagonism effects.

Table 1: Some examples of two or more plant extracts combined together and the type of interactions which they cause.

Latin name of plant extracts used	Part of plant used	Fungal organism(s) used	Type of interaction	Reference
Zuccagnia punctata with *Larrea cuneifolia*	Aerial parts ethanolic extract (both)	*Candida albicans, C. glabrata* and *Saccharomyces cerevisiae*	Synergisim	Moreno *et. al.*, 2020
Zuccagnia punctata with *Larrea nitida*	Aerial parts ethanolic extract (both)	*Candida glabrata*	Synergisim	Moreno *et. al.*, 2020
Zuccagnia punctata with *Larrea divaricate*	Aerial parts ethanolic extract (both)	*Candida glabrata*	Synergisim	Moreno *et. al.*, 2020
Annona senegalensis	Leaves ethanolic extract with twig aqueous extract	*Candida albicans, C. parapsilosis, C. neoformans* and *C. krusei*	Antagonisim	Bakarnga-Via *et. al.*, 2016
Annona senegalensis	Stem aqueous extract with twig aqueous extract	*Candida albicans, C. parapsilosis, C. neoformans* and *C. krusei*	Antagonisim	Bakarnga-Via *et. al.*, 2016
Annona senegalensis	Leaves ethanolic extract with bark	*Candida albicans, C.*	Antagonisim	Bakarnga-Via *et. al.*, 2016

	aqueous extract	*parapsilosis, C. neoformans* and *C. krusei*		
Annona senegalensis	Stem aqueous extract with bark aqueous extract	*Candida albicans, C. parapsilosis, C. neoformans* and *C. krusei*	Antagonisim	Bakarnga-Via *et. al.*, 2016
Zuccagnia punctata with *Larrea nitida*	Aerial parts dichloromethane extract	*Candida albicans* and *C. glabrata*	Synergisim	Butassi *et. al.*, 2015
Baccharis glutinosa with *Jacquinia macrocarpa*	Aerial parts ethyl acetate fraction of methanol extract with aerial parts n-butanol fraction of methanolic extract	*Aspergillus flavus* and *Fusarium verticillioides*	Synergisim	Medina-López *et. al.*, 2016
Dissotis multiflora with *Paullinia pinnata*	Leaves alcoholic extract with leaves alcoholic extract	*Candida krusei* and *C. albicans*	Synergisim	Ngandeu *et. al.*, 2019

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, they were *Saccharomyces cerevisiae, Candida albicans, C. glabrata, C. tropicalis, C. parapsilopsis* and *C. krusei*, the organisms were obtained from vaginal exudates of infected patients. The MIC values were determined for each extract, either alone or in combination between them; the FIC index was also calculated. According to the results report, "the synergist effect was observed with the combination between *Z. punctata / L. cuneifolia* against *C. albicans, C. glabrata*, and *S. cerevisiae*; also 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 both *C. albicans* and *C. tropicalis* (FIC index > 0.5 in both cases). Some combinations revealed indifferent interaction, in which the FIC indices were varying from 1.0 to 2.5. The most less active mixture was *T. andina / L. nitida*, which showed indifferent effect against all yeasts (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 tested strains, indicating that the interaction between chemical components contained in both plant species extracts is more effective as 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 some pathogenic yeasts. The MIC of all extracts on the yeasts were ranging from 0.156 to > 5 mg/ ml. *Candida krusei* was the most sensitive yeast while *Candida parapsilosis* and *Cryptococcus neoformans* were the most 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 tested yeasts (*Candida albicans, Candida parapsilosis, Candida krusei* and *Cryptococcus neoformans*), with MIC values ranged between 0.312 mg/ ml and 2.5 mg/ ml. The active extracts which exerted broad spectrum antifungal activity were considered for combination studies. The FICI of the combinations of LEtOH/ TwH_20, StH_20/ TwH_20, LEtOH/ BH_20 and StH_20/ BH_20 were varied from 5.50 to 48.09 on the four tested yeast strains, exhibiting antagonistic interactions (i.e. FICI> 4); the combinations led to 2- 4 fold reduction of antifungal activity as compared to the 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*. 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 agents" (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* was inactive against all the strains up to a concentration of 1,000 mg/ ml (MIC), 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 both extracts 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, they were *C. albicans, C. tropicalis, C. krusei, C. glabrata, C. dubliniensis* and other *Candida* spp isolated from denture wearers. Three different concentrations were prepared from the tested plants 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 was reported as zones of inhibition; all plant extracts showed remarkable antifungal activity except the aqueous extract of *C. sinensis* against almost all of the tested *Candida* strains. The best anti-candidal activity was found with the alcoholic extract of *N. sativa* (mean value = 12.3 mm), followed by the essential oil of *F. vulgare* (mean value = 7.9 mm); the results also exhibited that all mixed herbal extracts ranging from 7.8 to 15 mm, 7.6 to 15.5 mm and 7 to 15 mm inhibition zones for number (i), (ii) and (iii), respectively. The highest inhibition zone was related to mixture number (ii) (mean value = 12.3 mm), followed by mixture number (i) (mean value = 12.1 mm) and finally mixture number (iii) (mean value = 10.8 mm). Although lower concentrations of *N. sativa* along with higher concentrations of *F. vulgare* led to lowest antifungal activity of herbal mixtures, but there were no significant differences in action between 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 sequentially fractionated with hexane, ethyl acetate and n-butanol after being suspended in water. The ethyl acetate fraction of *B. glutinosa* and the n-butanol fraction of *J. macrocarpa* were mixed together and were tested for antifungal activity against *Aspergillus flavus* and *Fusarium verticillioides*, because these fractions were individually active. 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 the combination against *A. flavus* and *F. verticillioides* were 0.5272 and 0.4577 respectively, indicating a synergistic effect against both species. The synergistic effect was even seen at lower concentrations than those of the individual fractions. Only 12% and 8% of *A. flavus* and *F. verticillioides* spores treated with the synergistic mixtures respectively, were able to germinate (Medina-López *et. al.*, 2016).

The leaves of *Monodora tenuifolia (*Annonaceae), *Terminalia catappa* and *T. mantaly* (Combretaceae) were extracted 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.* Hot water, cold water and dichloromethane/ methanol (1:1) were the solvents used in their extraction. The aqueous extracts of these plants were inactive when tested alone, the DCM/ methanol extract of *P. falcatus* showed the highest activity (77.77% inhibition) against A. *niger* while DCM/ methanol extract of *M. foetida* showed the highest activity (77.78% inhibition) against *C. albicans*. *Aloe* spp. was inactive against *A. niger*. The combination of extracts (including the inactive *Aloe spp.* extract against *A. niger*) showed antifungal activity against *C. albicans* and *A. niger*; but the antifungal activity of all combinations was similar to the activities of all individual extracts against *C. albicans*. On the other hand, against *A. niger*, the individual extracts of *T. asiatica* and *Aloe* spp. were comparatively lower than that of their mixture. Hence a mixed actions of antagonism, additive and synergism were observed in this assay (Odhiambo *et. al.*, 2009).

"The stem-bark of *Euphorbia abyssinica* (Euphorbiaceae) and the whole plant 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 most potent was *Coleus* spp. extract, as its effect on *C. albicans* and *T. mentagrophytes* cells showed that the 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 only. However, when *C.* spp. and *E. abyssinica* extracts were combined, they exhibited no synergistic interactions against *C. albicans, T. mentagrophytes* and *M. gypseum"* (Ebob *et al.*, 2019). "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 only. 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 synergistic against *E. floccosum* in the time kill assay, the combinations also showed synergy on *E. floccossum* only, it showed additive or antagonistic activity on the rest of the tested fungi. In the checker board assay, *E. abyssinica* and *Coleus* spp. extracts showed synergistic effect against *T. mentagrophytes*. Another synergistic effects were also observed with *M. gypseum* at four different combinations of *E. abyssinica* and *Coleus* spp extracts proportions, but *C. albicans* showed some significant level of antagonism to the various tested combinations" (Ebob *et al.*, 2019).

"The leaves of *Dissotis multiflora* (Melastomataceae) and leaves of *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. Generally, the inhibition zone diameters were ranging from 10.33 mm to 19 mm, while the MICs and MFCs of the actives extracts were ranged respectively from 0,78 to 12,5 mg/ ml and 1,56 to 25 mg/ ml. The combinations showed significant antifungal activity compared to those of the individual fractions, as 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).

Allium sativum (Alliaceae) and *Nigella sativa* (Ranunculaceae) were tested together and each one alone and compared with fluconazole alone 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 has no antifungal activity (up to 10% concentration), and when combined with *A. sativum* it has weak antifungal activity against *C. albicans* when compared to *A. sativum* alone. Again, *A. sativum* extract with *N. sativa* caused an increase in the size of the zone of inhibition against *C. albicans* when compared to fluconazole alone. The study concluded that *A. sativum* extract has significant effect on *C. albicans*, *N. sativa* doesn't has anti-candidal activity on *C. albicans*, the synergistic effect of *N. sativa* extract with *A. sativum* extract has less anti-candidal activity than *A. sativum* extract alone but more activity when compared with fluconazole alone (Salih, 2016).

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. Differential activity was observed 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 greatest activity than their individual use (86.42%), while 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 following table **(Table 2)** shows some studies which had tested a combination of a plant extract(s) with Known antifungal agents, and showed if there is any synergism, indifferent, additive or antagonism effects.

Table 2: Some examples plant extract(s) combined with known antifungal agents; and the type of interactions which they cause.

Latin name of plant extracts used	Antifungal agent used	Fungal organism(s) used	Type of interaction	Reference
Terminalia catappa aerial parts of hydro-alcoholic extract	Nystatin and ketoconazole (each alone)	*Candida albicans*	Synergism	Toghueo and Boyom, 2014
Allium sativum ethyl acetate extract	Amphotericin B	*Cryptococcus neoformans*	Synergism	Davis *et. al.*, 1994
Astronium urundeuva	Amphotericin B	*Candida albicans*	Synergism	Bonifácio *et. al.*, 2019

hydro-ethanolic extract				
Uncaria tomentosa stem barks water insoluble fraction of hydro-ethanolic extract	Terbinafine	*Candida krusei* and *Candida glabrata* clinical resistant strains	Synergism	Moraes *et. al.*, 2017
Uncaria tomentosa stem barks water insoluble fraction of hydro-ethanolic extract	Fluconazole	*Candida krusei* and *Candida glabrata* clinical resistant strains	Additive	Moraes *et. al.*, 2017
Ocimum basilicum leaves ethanol extract	Amphotericin B	*Cryptococcus neoformans* and *C. gattii*	Synergism	Cardoso *et. al.*, 2017
Ocimum basilicum leaves hexane fraction of ethanol extract	Amphotericin B	*Cryptococcus neoformans* and *C. gattii*	Additive	Cardoso *et. al.*, 2017
Rubus chingii fruits ethanolic extract	Fluconazole	Fluconazole-resistant *Candida albicans*	Synergism	Han *et. al.*, 2016
Acmella caulirhiza hexane extract	Amphotericin B	*Candida krusei* and *C. orientaris*	Synergism	Olwenya *et. al.*, 2019
Acmella caulirhiza hexane extract	Amphotericin B	*Candida albicans, C. duabushaemulonii, C. haemulonii, C. auris* and *C. famata*	Antagonism	Olwenya *et. al.*, 2019
Acmella caulirhiza hexane extract	Clotrimazole	*Candida albicans, C. krusei* and *C. orientaris*	Synergism	Olwenya *et. al.*, 2019
Acmella caulirhiza hexane extract	Clotrimazole	*Candida duabushaemulonii, C. haemulonii, C. auris* and *C. famata*	Antagonism	Olwenya *et. al.*, 2019

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* was 2,048 µg/ ml. After combination, the MIC_{90} of *S. marianum* and fluconazole was 128 µg/ ml. Therefore, the aqueous extract of *S. marianum* in combination with fluconazole was more potent *in vitro* when compared with each one alone (Fozouni and Palang, 2018).

The leaves, twigs and stem of *Uvaria angolensis, U. muricata* (Annonaceae) and *Terminalia catappa* (Combretaceae) were extracted by water and ethanol. The extracts were evaluated each alone and when combined with nystatin and ketoconazole against yeasts species isolated from HIV patients 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 (MIC = 1.56 mg/ ml, 0.78 mg/ ml and 0.78 mg/ ml on *Candida albicans, Cryptococcus neoformans* and *Candida parapsilosis* respectively). The most active extract was combined 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 and suggest that they could serve as potential sources of antifungal agents (Toghueo and Boyom, 2014).

An 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 *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 *A. sativum* extract and amphotericin B respectively. In combination study, *A. sativum* extract and amphotericin B showed synergistic effect, as the fractional inhibitory concentration indices were 0.5. 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 out against clinical isolates strains of *Candida albicans* and *C. glabrata*, minimum fungicidal concentration (MIC) was also calculated, and finally determination of the activity of the free extract in combination with clinically used anti-fungal agents. Individual 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 plant extract were used in combination with different dilutions of fluconazole, caspofungin and amphotericin B, a majority of combinations showed indifference effect, with the notable exemption of the combinations between the plant extract and amphotericin B against *C. albicans* which resulted in synergism, this will lead to an increase of activity and a reduce of

the side effects of amphotericin B, which is mainly nephrotoxicity (Bonifácio *et. al.*, 2019).

A flower of *Flos Rosa Chinensis* (Rosaceae) was extracted with ethanol 70%, the extract was used to examine the anti-fungal activity 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 activity with 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 three fractions obtained from the aqueous extract of *Acca sellowiana* (Myrtaceae) leaves was tested against resistant strains of non-albicans *Candida*. Its reversal of fluconazole resistance was also tested by combining it with the plant fractions. The anti-fungal activity of the three fractions (F1, F2 and F3) was tested at 500 µg/ ml by micro dilution method. *C. glabrata* showed the lowest MIC values (500– 3.90 µg/ ml), and among all fractions F2 was the most effective. Checkerboard assay was applied to determine the effect of the combination of the F2 fraction with fluconazole, the combination showed FICI of ≤ 0.5 (synergism) against the *C. glabrata* resistant isolates. This study suggests that the combination of F2 and fluconazole might be used as an alternative treatment for mucocutaneus infections caused by resistant strains of non- albicans *Candida* species (Machado *et. al.*, 2016).

Uncaria tomentosa (Rubiaceae) stem barks were extracted with ethanol 50%, and the extract was tested for *in vitro* synergism test of its water insoluble fraction in combination with fluconazole and terbinafine (FLZ and TRB), the organisms used were resistant non *Candida albicans* isolates. Both checkerboard method and micro-dilution technique were used in this study. The isolates were *Candida krusei* ATCC 6258, CK01, CK04 and *Candida glabrata* CG40039, CG10, RL02, RL03. The results indicated that TRB and FLZ when tested alone up to a 64 µg/ ml were unable to inhibit the growth of all isolated strains. But the addition of water insoluble fraction to TRB resulted in enhancement of the anti-fungal activity of TRB, the combined concentration ratio was 8:1.95 µg/ ml concentration ratio, this enhancement activity was revealed on CK6258 terbinafine resistant isolate (88%), while the same isolate with TRB and water insoluble fraction alone showed only 40.7% and 20% respectively); TRB and water insoluble fraction of the plant extract at concentration ratio 4:1.95 µg/ ml caused significant cell damage (79.52%) regarding the CK04 isolate, the same combination was able to induce a significant synergic effect on nearly all isolates. On the other hand, regarding FLZ resistant isolate of CK04, a cell damage of about 80% was noticed for TRB and water insoluble fraction combined in a concentration ratio of 1.95:8 µg/ ml, in that case the individuals alone showed only 50% of cell damage below using the same concentration ratio. To conclude, the combinations of the water insoluble fraction of the plant extract with either TRB or FLZ were able to reduce the MIC values (either additive or synergic effects were

clearly noticed in all tested isolates); while for the plant fraction combination with TRB showed synergistic effect in four different isolates (two *C. krusei* and two *C. glabrata*); but also for the combination of the plant fraction and FLZ was active only in three isolates (one *C. krusei* and two *C. glabrata*); in other isolates an additive effects were observed (Moraes *et. al.*, 2017).

Absolute ethanol was used to prepare an extract from leaves of *Ocimum basilicum* (Lamiaceae), then the extract was suspended in distilled water and fractioned successively beginning with n-hexane, dichloromethane, ethyl acetate and ending with n-butanol. Also an essential oil was obtained from the plant. All these plant products were examined for anti-cryptococcal activity against three clinical strains of *Cryptococcus neoformans* T444, *C. neoformans* H99 and *C. gattii* WM779; also combinations were prepared using amphotericin B in which the FIC index values were ranging between 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 the 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 a 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 values 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 combinations were synergistic, only the combination of amphotericin B with hexane fraction was additive (Cardoso *et. al.*, 2017).

Leaves of *Eugenia uniflora* (Myrtaceae) were extracted by maceration with 95% ethanol. The extract was then diluted by DMSO, then it was assayed for anti-fungal activity, either alone or combined with amphotericin B, mebendazole, nystatin and metronidazole against *Candida albicans*, *C. krusei* and *C. tropicalis*. The MIC was >1,024 µg/ ml. However, an interesting potentiation of the anti-fungal activity was demonstrated when *E. uniflora* alcoholic extract was combined with metronidazole against *C. tropicalis*, as it lowered the MIC four times (from 128 to 32), no synergistic activity against the other species was seen. The study concluded that *E. uniflora* 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 against fluconazole-resistant *Candida albicans*. The growth curves for *C. albicans* after treatment with *R. chingii* extract, fluconazole alone and a combination were all constructed. Both *R. chingii* extract and fluconazole alone didn't show significant anti-fungal activity, but the two drugs when combined together showed significant synergy; the MIC_{80} for fluconazole was >256 mg/ ml and for *R. chingii* extract was >5,000 mg/ ml; the MIC_{80} for both combined drugs was only 0.0625– 16 mg/ ml for fluconazole and 4.88– 312.5 mg/ ml for *R. chingii* extract (Han *et. al.*, 2016).

The aerial parts of *Sarcococca saligna* (Buxaceae) plant was percolated with absolute ethanol. The antifungal activity was determined by disk diffusion method, for *S.*

saligna extract and its combination effect with fluconazole, the tested organisms were *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 indicates that these test strains were resistant to fluconazole. The *S. saligna* extract showed anti-fungal activity (MIC ≥ 0.5 mg/ disk) against *A. niger* and *A. treus*, the anti-fungal activity was dose-dependent. But S. *saligna* extract did not show any activity against *A. flavus* at contents used for the bioassay (0.5, 1, 2, 3 and 4 mg/ disk), also another tested strain (*A. fumigates*) was less susceptible to *S. saligna* extract compared with *A. niger* and *A. treus*. 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 investigated, it was reported that 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 methanol 80%. The extracts were then studied for their anti-candidal activity of each extract alone and in combination with either fluconazole 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 dissolved in DMSO 50% and their MIC values against *C. albicans* were established as 250 mg/ ml and 31.5 mg/ ml for twigs and leaves extracts respectively, unexpectedly, the growth of the blood isolated strain of *C. glabrata* was inhibited by the twigs extract at a relatively low concentration (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 fluconazole and caspofungin were decreased, so these extracts preparations increased fluconazole activity against both *C. albicans* and *C. glabrata*, thus the plant extracts 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 was studied against *Candida albicans* by the agar well diffusion method. Also the combination of this plant extract with amphotericin B was studied as equal volumes (25 µl) of each was added in the well and the zone of inhibition was measured. The results revealed that the plant extract had not showed any inhibition at concentration of 10 mg/ ml and 30 mg/ ml, but at the same concentrations, amphotericin B was susceptible against *C. albicans*. When the plant extract was combined with amphotericin B, the zone of inhibition had been increased significantly. The study concluded that the enhancement of anti-fungal activity of amphotericin B could be explained by the presence of biologically active compounds which are present in the plant extract. Thus, the combination of *T. chebula* extract with amphotericin B could be beneficial to increase the 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 antifungal activity of terbinafine is enhanced when used in a combination treatment with *M. oleifera* and *P. anisum* extracts (Khazia and Al-Janabi, 2019).

The leaves of *Vernonia adoensis* (Asteraceae) was successively extracted by hexane, dichloromethane, ethyl acetate, dichloromethane/ methanol, ethanol, methanol and water. All extracts were tested against inhibiting the growth of *Candida krusei*. The effects of combining fluconazole and the most potent extract were also examined. The MIC of fluconazole against *C. albicans* was found to be 8 µg/ ml, while its MIC against *C. krusei* was 125 µg/ ml. The MFC for fluconazole on C. *krusei* was also 125 µg/ ml, so, *C. krusei* was somehow resistant and less sensitive to fluconazole when compared with *C. albicans*. Therefore, subsequent work was then conducted on *C. krusei* only. The results for the effect of all tested *V. adoensis* extracts imply that all extracts (except the water extract) had no effect on inhibiting the growth of *C. krusei*, as the cell densities were still high. Hence distilled water extract of *V. adoensis* significantly reduced the fungi growth, so it was combined with fluconazole to determine if there was any enhanced effects, the concentrations of fluconazole were ranged between 500 µg/ ml to 8µg/ ml, and the concentrations of water extract were ranged between 100 µg/ ml to 12.5 µg/ ml, it was observed that the MIC of the combination of 100 µg/ ml of the water extract with 32 µg/ ml of fluconazole was achieved, as the combination lowered the MIC of fluconazole on *C. krusei* from 125 µg/ ml to 32 µg/ ml, also it was observed that the cell densities of the fungi decreased as the concentration of water extract increased, and decreased with a greater decrease observed at higher concentrations of fluconazole. The study concluded that combining different concentrations of fluconazole with 100 µg/ ml of the water extract increased the potency of fluconazole (Nyamuriya *et. al*., 2018).

A randomized controlled clinical trial was applied to determine the effect of *Salvia officinalis* (Lamiaceae) extract as vaginal tablets alone, and its effect in combination with clotrimazole on the recovery of vulvo-vaginal candidiasis and finally to compare its effectiveness. 111 participants were randomly divided into three groups of 37 patients using block randomization with block sizes of 6 and 9, and allocation ratio of 1:1:1, group one was treated with 100 mg vaginal tablet of clotrimazole and placebo (CP), group two was treated with 400 mg vaginal tablet of *S. officinalis* extract and placebo (SP), and group three was treated with vaginal tablet of *S. officinali*s extract and clotrimazole (SC), all once daily for 7 days; on the seventh day, vulvo-vaginal candidiasis was examined by vaginal symptoms and wet test, and if positive, it was 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 third group taking *S. officinalis* and clotrimazole than the reference second 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).

The dried aerial parts of *Echinophora platyloba* (Apiaceae) were macerated by ethanol 70%. Three different concentrations of the ethanolic extract were prepared (4, 5.2, and 11%). The antimicrobial and anti-fungal activities of each concentration alone was evaluated against *Candida albicans* by agar dilution and micro broth dilution assays. 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 also observed. *C. albicans* growth was inhibited by concentrations ≥ 2 mg/ ml of the plant extract (2, 4, 8, 16, 32, 64, 128 and 256 mg/ ml), also 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. Therefore, the most potent

anti-fungal agent was the mixture of ethanolic extract 5% plus amphotericin B, followed by amphotericin B alone, ethanolic extract 5% alone, ethanolic extract 11% alone, ethanolic extract 4% and lastly ethanol 70% in descending order. It was clear that *E. platyloba* showed 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, the study suggested that the synergistic effect of this mixture needs 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 (i.e. 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 out of four strains of (i.e. *A. flavus* and *A. terreus*) were sensitive to itraconazole, while the other two strains were resistant; the results also showed that all four strains were resistant to voriconazole. The aqueous and methanol extracts of green tea did not showed anti-fungal activity when tested individually, but the aqueous extract of green tea when combined with itraconazole were synergistic 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 of valuable effect. The study concluded that there is a valuable synergistic effects between the tested anti-fungal drugs and both 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 was done by plate antimicrobial susceptibility testing method. Checkerboard assays were applied to find the interaction of *H. sabdariffa* extract with fluconazole and voriconazole. When the extract was tested alone, the MIC values were ranged from 0.5 to 2 mg/ ml. All the isolated strains showed resistance to fluconazole (MIC > 16 µg/ml). All the isolated strains showed susceptibility to voriconazole with MICs values of < 0.016 µg/ ml. The results from the checkerboard assay indicate that the combinations of fluconazole with the extract have an 'indifference' effect; generally, the results revealed that combinations of fluconazole and *H. sabdariffa* have no synergism except for one strain among the six tested strains. In the case of variconazole, when the extract was combined with voriconazole at different concentrations, there was a reduction of the MIC values of voriconazole, in almost all of the six strains, there was a strong synergistic effect demonstrated by FICI calculations (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 (i.e. *Candida albicans*, *C. tropicalis*, *Trichosporon* spp, *Aspergillus fumigatus*, *A. flavus* and *A. versicolor*)

either alone or in combination between them. The result revealed 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 their crude ethanolic extracts. The MIC values of active fractions were ranged between 0.195 and 6.25mg/ ml, whereas the fungicidal activity was ranged between 0.781– 12.5 mg/ ml. The most efficient anti-fungal activity was showed 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 the most active fractions with fluconazole were tested by using well diffusion assay, the majority of combinations between one plant extract with another plant extract showed synergistic effect (for example petroleum ether fraction of *B. tournefortii* with *A. halimus*); also the majority of combinations between plant extracts and fluconazole showed even 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 a strong synergistic effect against *C. albicans* and *C. tropicalis*, a slight synergistic effect against *Tricosporon* spp and *A. versicolor*, and lastly antagonistic effect against *A. flavus* (Ibrahim *et. al.*, 2018).

The leaves of *Chromolaena odorata* (Asteraceae) were macerated in chloroform. The extract was then individually evaluated for its anti-fungal activity against *Aspergillus niger* using agar cup diffusion technique; while checkerboard technique was applied for evaluation its after combination with itraconazole. The results displayed that *A. niger* was sensitive to itraconazole (MIC = 0.0002 mg/ ml) and also sensitive to the chloroform extract of *C. odorata* (MIC = 5 mg/ ml). Their combination revealed that some concentration ratio showed additive properties, while some other ratios were synergistic, also indifferent properties were seen in some concentration ratios; the synergistic effect of *C. odorata* leaf chloroform extract and itraconazole was recorded in 4 ratios (i.e. 9:1, 8:2, 7:3 and 5:5), the ratio 5:5 produced the most synergistic effect. In the drug ratio that showed the greatest synergy (5:5), the MIC values of itraconazole and *C. odorata* extract were reduced by 20 and 8 times respectively, the interpretation is that *C. odorata* chloroform extract modified itraconazole activity to a much larger extent than does itraconazole alone (Ohadoma *et. al.*, 2016).

The aerial parts of *Tanacetum vulgare* (Asteraceae) was extracted by ethyl acetate at room temperature. The anti-fungal activity 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 displayed as size of zone of inhibition. The inhibition zone of chlorhexidine was 30.3– 19.3 mm, but in combination with ethyl acetate extract of *T. vulgare* (100 mg/ ml) the inhibition was from 32.7– 30 mm, indicating that this combination exerted a marked synergistic effect against *C. albicans*. In addition, the inhibition zone of sodium hypochlorite was between 69.7– 65 mm, which 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 some selected natural products from plants was applied against some fungal dermal infections which were resistant with some antifungal agents, the antifungal creams used were clotrimazole, fluconazole,

ketoconazole and terbinafine, they were combined with either turmeric rhizomes essential oil (*Curcuma longa*, Zingiberaceae) or *Aloe vera* (Asphodelaceae) gel; the tested 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 in this study revealed zones of inhibition with values ranged from 5 to 14.3 mm, the *C. longa* essential oil alone was 5 to 11 mm, while *Aloe vera* gel alone was ranged from 8 to 11.7 mm; the MIC values of antifungal creams, *C. longa* essential oil and *Aloe vera* gel were between 1.25 to 10 mg/ml. the combination of antifungal creams with either *C. longa* essential oil or *Aloe vera* gel revealed synergistic and indifferent properties (i.e. clotrimazole + *C. longa* E.O. against *C. albicans*, ketoconazole + *C. longa* E.O. against *A. niger*, terbinafine + *C. longa* E.O. against *C. albicans*, clotrimazole + *Aloe vera* gel against *C. albicans*, fluconazole + *C. longa* E.O. against *A. flavus* and terbinafine + *Aloe vera* gel against *C. tropicalis*), all displayed synergistic properties, while other combinations were indifferent without antagonism (Ogidi *et.al*, 2021).

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

The following table **(Table 3)** shows some studies which had tested a combination of a plant's essential oil with Known antifungal agents, and showed if there is any synergism, indifferent, additive or antagonism effects.

Table 3: Some examples of a plant's essential oil combined with known antifungal agents; and the type of interactions which they cause.

Latin name of plant's essential oil used	Antifungal agent used	Fungal organism(s) used	Type of interaction	Reference
Melaleuca alternifolia	Clotrimazole	19 strains of *Malassezia pachydermatis* isolated from healthy dogs	Synergism	Bohmova *et. al.*, 2019
Mentha piperita	Clotrimazole			
Origanum vulgare	Clotrimazole			
Cinnamomum cassia	Clotrimazole	19 strains of *Malassezia pachydermatis* isolated from healthy dogs	Indifferent	Bohmova *et. al.*, 2019
Syzygium aromaticum				
Melaleuca alternifolia	Clotrimazole	Redference strain of *Malassezia pachydermatis*	Additive	Bohmova *et. al.*, 2019
Syzygium aromaticum				
Melaleuca alternifolia	Nystatin	Some *Candida* species	Additive	Rosato *et. al.*,2009

Thymus broussonetii	Fluconazole	*Candida albicans*	Synergism	Saad *et. al.*, 2010
Thymus maroccanus				
Agastache rugosa	Ketoconazole	*Aspergillus niger, A. flavus, Blastoschizomyces capitatus, Candida albicans, C. utilis, C. tropicalis, Cryptococcus neoformans, Trichoderma viride, Trichophyton tonsurans* and *Trichosporon mucoides*	Synergism	Shin and Kang, 2003
Coriandrum sativum	Amphotericin B	*Candida albicans*	Synergism	Silva *et. al.*, 2011
		Candida tropicalis	Additive	

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. The combinations of clotrimazole with either *M. alternifolia*, *M. piperita* or *O. vulgare* essential oils displayed synergistic effect. The combinations of clotrimazole with either *C. cassia* or *S. aromaticum* essential oils showed indifferent effect. The combinations of clotrimazole with either *S. aromaticum* or *M. alternifolia* essential oils revealed an additive effect (Bohmova *et. al.*, 2019).

An essential oil from *Myrtus communis* (Myrtaceae) was evaluated for anti-fungal activity individually and in combination with amphotericin B against *Candida albicans* and different species of *Aspergillus* spp (i.e. *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 respectively for *C. albicans*, and their values were 4- 8 and 8 mg/ ml respectively for *Aspergillus* spp. Even the MICs of the tested plant's essential oil did not differ so much, as the MIC and MLC values were ranged between 8- 16 and 16- 32 ml/ ml respectively against the tested fungi. Checkboard micro titer test was used to examine the antifungal activity of amphotericin B with the

essential oil, it was observed that the MIC value of amphotericin B against *C. albicans* was lowered from 2 to 0.06 mg/ ml after the addition of essential oil at dose of 4 ml/ ml; the FIC values of the essential oil combined with amphotericin B were 0.25 ml/ ml and 0.03 mg/ ml for amphotericin B alone against *C. albicans*, while against *A. niger* were 0.25 ml/ ml and 0.015 mg/ ml respectively, the FIC index showed marked synergism against both *C. albicans* and *A. niger* (Mahboubi and Bidgoli, 2010).

The essential oils of the following plants were tested [*Mentha piperita* (Lamiaceae), *Carum copticum* (Apiaceae), *Cinnamomum verum* (Lauraceae), *Syzygium aromaticum* (Myrtaceae), *Cymbopogon martini* (Poaceae) and *Thymus vulgaris* (Lamiaceae)] and their active compounds were also tested (such as thymol, cinnamaldehyde, eugenol and geraniol), 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 examined essential oils were active (MIC range: 72– 288 µg/ ml; MFC range: 144– 576 µg/ ml). The essential oil of *M. piperita* was strongly active against *A. fumigatus* and moderately active against *T. rubrum* (MIC value = 288 and 576 µg/ ml respectively). Cinnamaldehyde, eugenol and geraniol showed antifungal activity higher than essential oils (MIC values ranged from 40- 160 µg/ ml, and MFC values ranged from 80- 320 µg/ ml); cinnamaldehyde was the most active against *A. solani* and *T. rubrum*. 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. Cinnamaldehyde was the most effective in combination therapy as it reduced the MIC of fluconazole up to 8 fold against both *A. fumigatus* and *T. rubrum*. 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 (Khan and Ahmad, 2011).

A study was conducted to test fluconazole against 32 clinical strains of fluconazole-resistant *Candida albicans* after their exposure to sub-lethal concentrations of tea tree oil (TTO) distilled from *Melaleuca alternifolia* (Myrtaceae) leaves or its main active constituent terpinen-4-ol using broth macro dilution. *C. albicans* strains tested to fluconazole alone were resistant but the same strains were sensitive to low concentrations of TTO. The MIC values of fluconazole were ranged between 64- 256 μg/ ml (average = 244 ± 47.22 μg/ ml). The MIC values of (TTO) were ranged from 0.06% to 0.5% (average = 0.19 ± 0.09%). Exposure of these strains for 24 hours to only 1/4 MIC of TTO and fluconazole (in combination) enhanced fluconazole activity, generally, 62.5% of isolates were classified as susceptible, 25% exhibited intermediate susceptibility and only 12.5% of strains were resistant. Also terpinen-4-ol strongly enhanced fluconazole activity against fluconazole- resistant *C. albicans* strains, the MIC values of terpinen-4-ol ranged from 0.06% to 0.25% (average = 0.11 ± 0.09%); exposure of these strains for 24h to fluconazole and only 1/4 MIC of terpinen-4-ol strongly enhanced fluconazole activity, and all of *C. albicans* isolates were classified as susceptible (Mertas *et. al.*, 2015).

The anti-candidal activity of eugenol (main component of clove oil) and thymol (main component of thyme oil) either alone or in combination was evaluated against the architecture shape of the envelope of *C. albicans*. All 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 eugenol or thymol induced a dramatic change in the morphology of the envelope. Also it was found that thymol proved to be about 40– 50% more active than eugenol. On the other hand, the combination of 1 MIC of eugenol plus 1 MIC of thymol induced a significant increase in the number of damaged cells in comparison with the corresponding single concentrations of both molecules. The study concluded after measuring the MIC values of both molecules combined together in different incubation times, the presence of a synergistic effect (Braga *et. al*., 2007).

A study was conducted to evaluate the antifungal activity of essential oil of *Ocimum basilicum* (Lamiaceae) leaves and its major components (i.e. linalool and geraniol), the species used in this study were both fluconazole sensitive and resistant strains of *Candida albicans* and *Cryptococcus neoformans*. The results showed that all combinations produced FIC index values ranging from 0.3826 to 0.6326, also all these combinations significantly reduced their MIC values. The synergistic effect was observed in the combination of flucoconazole and geraniol (MIC reduced from 31.25 to 4.14 μg/ ml and 76 to 19 μg/ ml respectively), and in the combination of linalool with geraniol (MIC values decreased from 790 to 111 μg/ ml and 76 to 19 μg/ ml respectively). It was also obviously seen that the concentrations needed from the two combined components to completely eradicate *C. neoformans* were very low. One interesting point was there was no synergistic effect in the combinations of natural components with flucoconazole against *C. albicans* sensitive strain. However, a synergistic effect was observed in the combination of linalool with geraniol (MIC values reduced from 790 to 105 μg/ ml and 152 to 38 μg/ ml respectively). Furthermore, all combinations tested showed synergistic effect against *C. albicans* resistant strain. Flucoconazole was also combined with the essential oil at concentration 156 μg/ ml, it was found that the 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 (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. Micro dilution method was used to determine MIC and FIC values. The MIC of *O. vulgare* essential oil alone, MIC of *O. vulgare* essential oil with nystatin and the FIC of nystatin were 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 respectively. The *P. graveolens* essential 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 were obtained with *P. graveolens* and few

results were 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. *O. vulgare* essential oil was the most effective among the essential oils. Some combinations of nystatin and *P. graveolens* essential oil did not have any synergistic effect 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 of Moroccan *Thymus broussonetii* and *T. maroccanus* (Lamiaceae) with amphotericin B and fluconazole were tested against *Candida albicans*. Macro dilution broth method was used. Most of the essential oils showed significant anti-candidal activity with MIC of about 0.25 mg/ ml. Chekerboard titer assay was applied after combining the essential oils with either amphotericin B or fluconazole, the FICIs revealed significant decrease in the MIC values of the individuals, for example, the MIC of amphotericin B alone was lowered from 16 to 4 g/ ml in the presence of *T. maroccanus* essential oil. Synergistic effects were obtained after using different combinations of *T. maroccanus* and *T. broussonetii* essential oils with either amphotericin B or fluconazole, as their FICI values were ranged between 0.27- 0.49. The results indicate that the synergistic effect of essential oils with fluconazole was stronger than the combination with amphotericin B. The study suggested that the use of these combinations are likely to reduce the minimum effective dose of the drugs, and hence minimizing their side effects and their treatment costs (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 the MIC value of estragole in most tested fungi. This finding suggests that the activity of the essential oil is probably based on estragole component, which makes up half of the essential oil content, while the other constituents have relatively mild activity. Ketoconazole had much higher activity than either estragole or *A. rugosa* essential oil with MIC values ranging between 12.5- 25 µg/ ml. When ketoconazole was combined with estragole it caused a significant decrease in the MIC value compared with each one alone. The isobologram constructed confirmed that 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 (Shin and Kang, 2003).

The anti-candidal effect of the essential oils of *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 essential oils of *Thymus vulgaris* thymol chemotype gave the strongest inhibitory effect (thymol content is 63.22% of the essential oil), and because of its promising activity it was studied in combination with amphotericin B;

the results displayed that low concentrations from the essential oil produced a high increase in the MIC 80%, the strongest increase being obtained with a concentration of essential oil equal to 0.0025 µg/ ml. 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% (i.e. 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). At concentrations of 0.2 and 0.3 µl/ ml the essential oil showed a decrease of the MIC 80% of amphotericin B compared with those of amphotericin B alone. The strongest decrease (48%) was achieved in medium containing 0.2 µl/ ml of the essential oil, while an essential oil concentration of 0.3 µl/ ml gave a total inhibition of the fungal growth with 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 of *Cinnamomum cassia* (Lauraceae) either alone or combined with amphotericin B were investigated against *C. albicans*. The composition of the oil was analysed by GC/MS and showed high content of cinnamaldehyde (92.2%). Macro broth dilution method was applied to determine the MIC 80%. The results showed an increase of MIC values with essential oil concentrations ranging between 0.08- 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 enhancement of amphotericin B activity may contribute for the development of less toxic and more effective therapies, especially in treatment of candidiasis associated with HIV infection (Giordani *et. al.*, 2006).

Essential oils obtained from *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, displayed significant antifungal activity, the MIC values of the essential oil of *C. martini* against *Microsporum gypseum* and *Trichophyton rubrum* were 200 and 150 ppm respectively, they were comparatively less than the MIC values of the essential oil of *C. ambrosioides* against *M. gypseum* (700 ppm) and *T. rubrum* (350 ppm). After combination, the MIC values of both essential oils were also less than that of *C. ambrosioides* against *M. gypseum* (i.e. 500 ppm) and *T. rubrum* (i.e. 250 ppm). The MLC values of the essential oils and their combinations were ranged from 500 to > 1,000 ppm against the dermatophytes. *T. rubrum* was found to be the most sensitive against the essential oils. On the other hand, the MIC values of griseofulvin, ketoconazole and fluconazole were between 1,000- 5,500 ppm,

which are much greater than the MICs of the essential oils and their combinations (i.e. 150–700 ppm). In *in vivo* study, the essential oil ointments were 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; all the essential oil ointments were effective (*C. martini* > essential oils combinations > *C. ambrosioides*) in a time-dependent manner, the essential oil of *C. martini* showed complete cure of *T. rubrum* and *M. gypseum* infections at day 17 and day 21 respectively, while the essential oil of *C. ambrosioides* and its oil combinations cured the disease in most of the treatment models at day 21. The study concluded that the essential oils of both species are recommended in treatment of dermatophyte infections, and may applied as an alternative to synthetic drug for topical application because of their activity and synergism (Prasad *et. al*., 2010).

The antifungal activity of *Coriandrum sativum* (Apiaceae) essential oil either alone or its combination with amphotericin B was studied against two strains of *Candida albicans* and one strain of *C. tropicalis* by using micro dilution broth susceptibility assay and Checkerboard assay, respectively. The records represented that *C. sativum* essential oil has a fungicidal property with MLC values equal to the MIC value and ranging between 0.05- 0.4% (v/v), the fungicidal property was a result of cytoplasmic membrane damage and subsequent leakage of intracellular components such as DNA. Also, concentrations bellow MIC value caused obvious reduction in the percentage of germ tube formation in *C. albicans* strains. A synergetic effect was seen against *C. albicans* strains after applying *C. sativum* essential oil and amphotericin B together, while additive effect was seen against the essential oil of *C. tropicalis* (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*) were investigated and compared with the activity of allicin. The fungistatic activities of *Allium* species essential oils, allicin and ketoconazole among others were singly evaluated by broth dilution method and disk diffusion assay. From the results, *A. sativum* for. *pekinense* essential oil was the most potent inhibitor of all three *Trichophyton* species, with MIC values of about 64 mg/ ml, equivalent to 25–50% of the activity of allicin (i.e. 16– 32 mg/ ml). The combinations of either *A. sativum* essential oil or allicin with ketoconazole were tested by the checker board titer test, the FICI values were ranged between 0.09- 0.12, it showed significant synergism of ketoconazole with *Allium* species. Moreover, the combination of Ketoconazole with allicin resulted in additive effects, with FICI values between 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 ketoconazole. Among the essential oil fractions tested alone, *Cymbopogon citratus* leaf (Poaceae) and *Eucalyptus globulus* leaf (Myrtaceae) were the most potent, with MIC values of < 0.125– 0.25 mg/ ml and MFC values of < 0.125– 1 mg/ ml. *Thymus vulgaris* (Lamiaceae) essential oil and its main component thymol, gave high MIC values ranging between 0.25 and 1 mg/ ml, thymol was more active than the total essential oil of *T. vulgaris.* The essential oil of *Pelargonium*

graveolens (Geraniaceae) as well as its main components (i.e. citronellol and geraniol) showed strong inhibition against these fungi, with MIC values between 0.25– 2 mg/ ml, and because of its strong effect, the combined effects between *P. graveolens* essential oil, citronellol and geraniol with ketoconazole were evaluated by using a checkerboard microtitre assay against *Trichophyton* spp, the MIC figures of ketoconazole when combined with *P. graveolens* essential oil were significantly lowered, with FIC indices ranging between 0.18 and 0.56. Moreover, in an experiment versus *T. erinacei* and *T. soudanense*, the FIC indexes of ketoconazole with citronellol or geraniol were 0.06 and 0.13 respectively. FIC indices indicate the strongest synergism between *P. graveolens* essential 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 again 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, also they were tested in combination with either itraconazole or amphotrericin B against clinical isolates of *Candidas albicans*. The most active samples were the essential oils of *P. bredemeyeri* (MIC range 157.5- 222.7 µg/ ml), *L. origanoides* (MIC range 157.5- 198.4 µg/ ml) and *M. royoc* (MIC = 250 µg/ ml). The most synergistic effect was observed for the combination of itraconazole with essential oil of *P. bredemeyeri* (FICI range 0.09- 0.13), but no interactions were detected for the combination of amphotericin B with essential oil of *P. bredemeyeri* (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 their flowering stage and their essential oils were obtained by hydro-distillation method. 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* essential oils showed broad spectrum activity against many pathogenic *Candida* strains. The synergistic property was exhibited with the combinations of *C. verum*/ fluconazole and *P. graveolens*/ fluconazole (FIC = 0.37), it was found 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 combinations with fluconazole causes disturbances in fatty acid homeostasis in cells of *C. albicans* as well as affecting their ergosterol biosynthesis, the quantity of ergosterol and oleic acid was reduced to 52.33% and 72% respectively (Essid *et. al.*, 2017).

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

The following table **(Table 4)** shows some studies which had tested a combination of a plant's isolated phytochemical compound with Known antifungal agents, and showed if there is any synergism, indifferent, additive or antagonism effects.

Table 4: Some examples of a plant's isolated phytochemical compound combined with known antifungal agents; and the type of interactions which they cause.

Name of isolated phytochemical compound used	Antifungal agent used	Fungal organism(s) used	Type of interaction	Reference
Carvacrol	Fluconazole	25 clinical isolates of *Candida auris*	Synergism	Shaban *et. al.*, 2020
	Amphotericin B		Additive	
	Nystatin		Synergism	
	Caspofungin		Additive	
Epigallocatechin gallate	Miconazole	*Candida albicans, C. parapsilosis, C. tropicalis, C. glabrata, C. kefyr* and *C. krusei*	Synergism	Ning *et. al.*, 2015
	Amphotericin B			
Baicalein (flavones)	Fluconazole	*C. parapsilosis*	Synergism	Serpa *et. al.*, 2012
Osthole (coumarin)	Fluconazole	Fluconazole-resistant *Candida albicans*	Synergism	Li *et. al.*, 2017
Eugenol,	Fluconazole	Resistant strains and biofilms of *Candida spp*	Synergism	Lu *et. al.*, 2017
Methyleugenol				
Magnolol (phenyl propanoid compounds)				
Farnesol (monoterpene compound)	Fluconazole	Resistant strains and biofilms of *Candida spp*	Synergism or additive	Lu *et. al.*, 2017
Thymol	Nystatin	*Malassezia pachydermatis*	Synergism	Schlemmer *et. al.*, 2019
Carvacrol				
Carvacrol	Miconazole			
Cinnamaldehyde	Fluconazole	*Malassezia pachydermatis*	Indifferent	Schlemmer *et. al.*, 2019
Thymol	Terbinafine			
Cinnamaldehyde				
Carvacrol	Ketoconazole	*Malassezia pachydermatis*	Antagonism	Schlemmer *et. al.*, 2019
Thymol				
Cinnamaldehyde				

A study was conducted to evaluate some anti-fungal agents in combination with some monoterpene phenols, the phenolic monoterpene compounds tested were carvacrol,

thymol, eugenol and methyl eugenol, while the tested anti-fungal agents were fluconazole, amphotericin B, nystatin and caspofungin, 25 clinical isolates of *Candida auris* were involved in this study. MIC results showed that all tested compounds have anti-fungal activity at varying levels, carvacrol gave the best MIC value (125 µg/ ml) followed by thymol (MIC = 312 µg/ ml). The MFC values for the all four tested compounds were 1– 2 folds higher than their respective MIC values. Carvacrol was combined with fluconazole, amphotericin B, nystatin and caspofungin, the results showed both synergistic and additive effects in 68%, 64%, 96% and 28% respectively. Therefore the study recommended that carvacrol has a potential to be developed into a novel anti-fungal agent against *C. auris* (Shaban *et. al.,* 2020).

A research was conducted to determine the anti-fungal activity of curcumin obtained from *Curcuma longa* (Zingiberaceae) and to examine its possibilities to be combined with fluconazole and itraconazole. The MIC of fluconazole, itraconazole and curcumin was found to be in a range of 32- 64 µg/ ml, 8- 32 µg/ ml and 64- 256 µg/ ml respectively. The results of the *in vitro* anti-fungal study was based on measuring the zones of inhibition of the prepared combinations at standard concentration of 10 µg/ ml, it was found that curcumin significantly increases the anti-fungal capacity of both fluconazole and itraconazole, and after calculating their FICIs, it was found that the increase in anti-fungal capacity was due to either 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 signs 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 against *S. cerevisiae* cells at 1.2 mM, whereas polymyxin B showed static activity at 60 µg/ ml. The organism cells were subjected to lethal damage when polymyxin B was combined with zwiebelane A, while the normal architecture of the vacuoles was maintained when the organism cells were treated with either polymyxin B alone (60 µg/ ml) or zwiebelane A alone (1.2 mM) (Borjihan *et. al.,* 2010).

Three saponins (i.e. ceposide A, ceposide B, and ceposide C) were isolated and extracted by acetone from the white onion bulbs, *Allium cepa* (Liliaceae), they were evaluated for their antimicrobial activity either alone or in selected 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*). The results displayed that the anti-fungal activity of the three saponins increases related with their concentrations (i.e. ceposide B > ceposide A > ceposide C). When these saponins were combined, additive effects were detected, however, significant synergism effect was detected after applying mixture of 33.3% for each ceposide compound against *B. cinerea* and *T. atroviride,* the growth of these two fungi organisms was strongly inhibited when saponins were applied in combination with *B. cinerea* 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 *Candida* species growth at concentrations ranging between 375- 1,500 mg/ ml. However, it showed synergistic property against many *Candida* spp after combination with miconazole, fluconazole and amphotericin B (FICI range between 0.15- 0.50). When EGCG was applied in combination with miconazole and amphotericin B, synergistic effect was seen against all species (The MICs of miconazole reduced from 0.25– 1 to 0.031– 0.25 mg/ ml, The 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 is an active compound which was obtained from the aerial parts of *Colebrookea oppositifolia* (Lamiaceae) which extracted by 50% hydro-ethanol. Acteoside and amphotericin B were examined each one alone and in combination against *Candida albicans, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis, Cryptococcus neoformans, Aspergillus flavus, A. fumigatus, A. niger* and *A. parasiticus* clinical isolates. The MIC values of amphotericin B were calculated in the absence and presence of increasing concentrations of acteoside (i.e. 0.195–12.5 mg/ ml) by using two-dimensional checker board micro broth dilution method. The interesting finds is acteoside (alone) did not showed any anti-fungal activity at concentrations up to 1,000 mg/ ml, but it showed synergistic property in combination with amphotericin B against all tested organisims (with FIC indices ranged between 0.0312– 0.1562). The fungicidal effect of the combination of amphotericin B with acteoside was again assessed on *C. albicans*, *A. fumigatus* and *C. neoformans*, amphotericin B was used at a concentration of 0.256 x MIC (i.e. 0.1256 x MIC against *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 action, whilst the fungicidal activity (99.9% kill) was achieved at 46 x MIC (i.e. 26 x MIC against *C. neoformans*) after 24 hours when compared with the growth of the 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 hours. 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 combinations against 64 fluoconazole-sensitive and 34 fluoconazole-resistant *Candida* clinical isolates was highlighted. FICI values were calculated from checkerboard micro dilution assays. The MIC values of eugenol and methyleugenol against *C. albicans, C. tropicalis* and *C. glabrata* were ranged between 475- 500 mg/ ml and 340- 350 mg/ ml respectively, while the MIC of fluoconazole was ranged between 2.5– 7.5 mg/ ml and was within the reference ranges. It was noted that the resistant to fluoconazole (i.e. MICs 80– 110 mg/ ml) showed sensitivity to both eugenol and methyleugenol. However, it is clear that

methyleugenol proved to be more active against all *Candida* species than eugenol. The anti-fungal activity of both compounds was increased with increasing of their concentrations. All the fluoconazole-susceptible and resistant *Candida* isolates showed high degree of sensitivity from their large inhibition zones. The FICI values for eugenol/ fluoconazole and methyleugenol/ flucoconazole combinations against all flucoconazole-sensitive isolates were ranged between 0.31- 0.55 and 0.24- 0.58 respectively, the interaction of eugenol/ flucoconazole combination was synergistic in 58 out from 64 isolates, whereas for methyleugenol/ flucoconazole it was 59, then only 5 isolates showed indifference property. Out of 34 flucoconazole-resistant strains, 29 and 31 isolates showed synergistic affects for eugenol/ flucoconazole and methyleugenol/ flucoconazole respectively. No antagonistic activity was seen in this study. The study recommended that fluoconazole can be supplemented with eugenol and methyleugenol to treat flucoconazole-resistant Candidiasis (Ahmad *et. al.*, 2010).

Evaluation of the *in vitro* activity of baicalein, a 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. Exposure to baicalein at MIC_{50} values obtained for each strain and at 260 mg/ ml resulted in significant anti-candidal activity. The anti-fungal activity of fluconazole/ baicalein combination was greater than the individual contribution of each agent, according to FICI values, the combination showed partial synergistic property against *C. albicans* and *C. tropicalis*, while the combination produced synergistic property against *C. parapsilosis*, in this case, the fluconazole MIC changed from susceptible dose-dependent (i.e. MIC_{50}= 516 mg/ ml) to susceptible (i.e. MIC_{50}= 50.125 mg/ ml). The study concluded that the combination of baicalein with fluconazole may represent an attractive prospect for the development of new strategies for treating 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 the purified catechins against *C. albicans* was observed at 125 mg/ ml, while its minimum fungicidal activity (MFC) was shown between 250- 1 mg/ ml. The MIC of Fluconazole was observed at concentrations of 64 mg/ ml and 128 mg/ ml for *C. albicans* and *C. glabrata* respectively, while the MIC value of Amphotericin B was observed at concentrations of 1 mg/ ml against both *Candida* species. Catechins showed synergistic effect with fluconazole and amphotericin B against *Candida* spp. Time kill assay also showed synergistic effect at MIC and twice of the MIC of purified catechins and it's combinations. Furthermore, copper sulphate increased the anti-candidal activity of the synergistic combinations by 0.4% to 6.63%. The study concluded that the promising anti-candidal activity requires further investigations of safety profile for 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 were applied (MIC_{50} greater than or equal to

8 µg/ ml), and 10 fluconazole-susceptible C. *albicans* isolates (MIC_{50} less than or equal 1 µg/ ml) were also involved in this study. The results showed that osthole alone did not showed anti-fungal activity (i.e. MIC_{50} was greater than 64 µg/ ml). The combination of osthole with fluconazole showed significant synergistic effect against the fluconazole-resistant *C. albicans*, the dose of fluconazole was reduced from 1 to 16 µg/ ml, and the dose of osthole was reduced from 4 to 16 µg/ ml, also the FICI 0.04 to 0.31, the synergistic effect was dose-dependent according to the growth curve assay. Unlike the result report of fluconazole-resistant isolates, both fluconazole and osthole did not displayed synergistic effect on fluconazole-susceptible strains, as the FICI was 0.51 to 2.01 (Li *et. al.*, 2017).

Anti-fungal activity of cinnamaldehyde, eugenol, honokiol, magnolol and shikonin was evaluated, either alone or in combination with fluconazole against some *Candida* species. When the phytocompounds were tested alone, some exhibited significant anti-fungal activity, with MICs of ≤ 8 µg/ ml, and other compounds were even more potent than fluconazole or itraconazole (i.e. honokiol, magnolol and shikonin). In the group of phenylpropanoids, some compounds demonstrated slight or moderate efficacy, such as cinnamaldehyde, eugenol and magnolol. However, in combination with fluconazole they showed significant synergistic effects, including resistant strains of *Candida* species. For instance, when eugenol, methyleugenol and magnolol were used in combinations with fluconazole, the FICI values revealed high synergism (FICI< 0.5). On the other hand, some terpenoids like farnesol were tested, it showed synergistic and additive effects with fluconazole against drug-resistant *Candida* isolates and *C. albicans* biofilms. (Lu *et. al.*, 2017).

Anti-candidal activity of two asarones (α and β) isolated from alcoholic extract of *Acorus calamus* (Acoraceae), were tested in combinations with either fluconazole, clotrimazole or amphotericin B, the tested organisms were *Candida albicans* and *C. tropicalis*. The highest activity was shown by β-asarone, with MIC values ranged between 64– 125 µg/ ml, while α-asarone showed the activity at MIC values ranged between 250– 500 µg/ ml, for azole drugs the activity was ranged between 1- 4 µg/ ml and for amphotericin B the activity was ranged only between 1- 2 µg/ ml. The combined anti-candidal activities of asarones and the chosen drugs were assessed by using checkerboard micro-dilution and time-kill assays, the results displayed significant synergistic effect, especially the combinations of β-asarone with azoles and amphotericin B. Antagonism and indifference effects were not recorded in all combinations, the MIC values have been reduced to more than 8 times in the combinations of α and β asarones with azoles and amphotericin B (Kumar *et. al.*, 2015).

β carbolines such as harman, harmine, harmaline and harmalol are pharmacologically active alkaloids which are present in the seeds of *Peganum harmala* (Nitrariaceae). These β-carboline alkaloids are extracted by methanol through bioassay-guided fractionation process and their anti-fungal activities were investigated either alone or in combinations against *Aspergillus niger* and *Candida albicans*. The isolated β-carboline alkaloids showed anti-microbial effects against all tested microorganisms as the diameters of zone of inhibition were ranged between 10.5 and 31.5 mm. When the alkaloids were examined individually, *C. albicans* was the most susceptible to harmine (22.2 mm), while harman was the most active against *A. niger* (20.8 mm).

Harmaline was more effective against *C. albicans* (21.3 mm), meanwhile, harmalol showed moderate activities. A combination of harman and harmaline mixture was active against *C. albicans* (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 either harmine or harmaline (Nenaah, 2010).

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

Hydrated catechin, hydrated quercetin and (-) epigallocatechin gallate were combined with fluconazole and examined against fluconazole-resistant *Candida tropicalis* strains. All strains had showed MIC_{50} value of 64 µg/ ml for flucoconazole. The flavonoids when tested alone had not shown any anti-fungal activity, but when they were used as a co-treatment with fluconazole, there was significant synergistic effect. The synergism between the flavonoids and fluconazole was determined by using checkerboard technique, the FICI values were ranged between 0.25- 0.38 µg/ ml (da Silva *et. al.*, 2014).

Plant latex – drug(s) combinations:
The following table **(Table 5)** shows studies which had tested a combination of a plant's latex exudate with Known antifungal agents, and showed a synergism effect.

Table 5: Examples of a plant's latex exudate combined with known antifungal agents; which showed synergism effect.

Latin name of plant's latex used	Antifungal agent used	Fungal organism used	Type of interaction	Reference
Euphorbia characias latex	Ketoconazole	*Candida albicans*	Synergism	Giordani *et. al.*, 2001
Carica papaya latex sap	Fluconazole	*Candida albicans*	Synergism	Giordani *et. al.*,1997
Hevea brasiliensis sap	Amphotericin B	*Candida albicans*	Synergism	Giordani *et. al.*, 2002

Euphorbia characias (Euphorbiaceae) latex production was initiated after making repeated cuts along the stems, and had been collected by Eppendorff tubes and stored at 4 ℃. The *in vitro* suseptibility of *Candida albicans* to ketoconazole and *E. characias* latex either alone for each or in combination of both was examined using macro-broth dilution method. The concentration of latex was estimated by its proteins

content via Bradford's method. The MIC 80% of the crude latex and ketoconazole were 159 µg protein/ ml and 0.3901 µg/ ml respectively. The examination of the mixture containing latex at several concentrations (i.e. 7.8, 15.62, 31.25, 62.5 and 125 µg protein/ ml) combined with ketoconazole indicates a synergistic effect; in the case of latex, after its concentrations of 31.25 and 62.5 µg protein/ ml were tested, it was found that the MIC 80% of ketoconazole were lowered to 0.194 and 0.183 µg/ ml respectively, as the MIC 80% of ketoconazole alone was 0.390 µg/ ml (Giordani *et. al.*, 2001).

Carica papaya (Caricaceae) latex sap (0.41 mg protein/ ml) in combination with 2 µg/ ml of fluconazole were examined against the growth of C*andida albicans*. The mixture showed synergistic action, this synergistic effect was due to partial cell wall degradation. When the concentration of fluconazole was increased to 4 µg/ ml, it was recorded that there is a small decrease of MIC 80% of the latex (i.e. from 150 to 130 µg protein/ ml) (Giordani *et. al*.,1997).

The *Hevea brasiliensis* (Euphorbiaceae) latex obtained from the rubber trees was treated with ammonia (to prevent rubber coagulation), amphotericin B was dissolved in 100% DMSO. The anti-fungal activity of the latex was tested with various fungal strains by using macro-broth dilution assays, also the MIC 80% was estimated singly and in combination with amphotericin B. In individual assay, it was concluded that the growth of all tested organisms was inhibited by *H. brasiliensis* latex, the strongest antifungal activity was achieved against *Trichosporon cutaneum* (i.e. MIC 80% = 40.615 µg protein/ ml) and *Cryptococcus neoformans* (i.e. MIC 80% = 56.078 µg protein/ ml). In the combination assay, *Candida albicans* was cultured on a medium supplemented with different concentrations of *H. brasiliensis* latex and amphotericin B combinations, the concentration of the latex was ranged between 7.5 to 60 µg protein/ ml, it was found that the best MIC 80% (0.201 µg amphotericin B/ ml) was observed when the culture medium contained 60 µg protein/ ml, the use of 15 and 30 µg protein/ ml gave MIC values that were slightly higher (0.221 and 0.247 µg protein/ ml respectively), also a great MIC 80% (0.369 µg amphotericin B) was observed with the addition of 7.5 µg protein/ ml in the culture medium. The study concluded that the MIC 80% decreases strongly for the latex concentrations in a range between 0– 15 µg protein/ ml, then decreases very slightly with higher concentrations up to 60 µg protein/ ml, amphotericin B showed synergistic effect with all tested *H. brasiliensis* latex concentrations, 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:

To conclude, many people are using combination therapy in treatment of many fungal infections, specially in developing countries, these combinations are either plant-plant combinations or plant product-antifungal drug combinations, many of these combinations are used in the practice of traditional medicine from ancient time (specially plant-plant combinations), these combinations lead to herbal-herbal or herbal-drug interactions, in which these interactions can be useful (synergistic effect) or may lead to a decrease in the overall antifungal activity (i.e. antagonistic effect). That is why many researches had be conducted to test and to prove the efficacy and safety of many combination preparations used as anti-fungal agent in traditional medicine, so the main aim of this review was to collect many researches which had

tested all of these combinations, and listed them in a form of synergistic, additive, indifferent or antagonistic effects, this will help many researchers to choose a combination preparations which have synergistic effect for further analysis, and to avoid preparations which have antagonistic effect. Moreover, our review showed that combinations with synergistic effect will offer significant opportunity to develop novel antifungal therapies, specially against resistant fungal organisms, this will decrease the opportunity to develop resistance and also reduce adverse effects for some current available antifungal agents. We recommend that further studies are needed to study the pharmacokinetic and pharmacodynamic profiles of these novel synergistic combinations.

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