

Assessment of Secondary Metabolites and Thin-Layer Chromatographic Analysis of *Carica papaya* (Caricaceae) Leaves Ethanolic Extract

ABSTRACT

Carica papaya, also referred to as pawpaw, is a tropical fruit-bearing tree in the Caricaceae family. Papaya is one of the world's most extensively grown crops. Its fruits, seeds, and leaves are widely utilized in cooking and traditional medicine. Papaya leaf extract, tea, and juices are extensively used to cure ailments and promote health. The study aims to discover and quantify the presence of potential chemical compounds in *C. papaya* leaves using Thin-Layer Chromatographic analysis (TLC). Standard qualitative phytochemical tests were performed on the leaves of *C. papaya* to determine the presence of various phytochemicals, and TLC analysis to ascertain the presence of potential chemical constituents. Extract of *C. papaya* leaves in ethanol showed the presence of tannins, alkaloids, glycosides, steroids, proteins, flavonoids, carbohydrates, etc., after the phytochemical screening, all of which are useful chemical ingredients responsible for a variety of pharmacological effects. The lack of color change noticed when the heated sample was treated with Fehling's solution revealed the absence of reducing sugar. TLC analysis using several solvent systems revealed various phytochemicals with varying R_f values. This variance in phytochemical R_f values showed the phytochemicals and potential chemical constituents' polarity and this will give a hint on the type of solvent systems suitable for the analysis and separation of pure components. As a result, *C. papaya* leaf extract contains a high concentration of both primary and secondary plant metabolites. TLC profiling yielded an incredible result, suggesting the existence of numerous possible chemical ingredients.

Keywords: *Carica papaya*, phytochemical analysis, Secondary Metabolites, Thin Layer Chromatography.

INTRODUCTION

Traditional herbal medicine development and utilization can be traced back to the Stone Age (Pan et al., 2014). Traditional healing and magic or incantations appear to be significantly more common than Western medicine (Natako, 2006). Traditional medicine in Africa is an accepted form of medical care, with some components in herbalism, divination, and spirituality (Chavunduka, 2017, Mahomoodally, 2017).

Herbal medicine is the utilization of herbal products, herbal supplements, or finished herbal products including whole plants, plant parts, such as leaves, bark, fruits and vegetable flowers, and roots, or their extracted active ingredients intended for human or animal medicinal use, according to the World Health Organization (WHO). According to reports, almost 80% of Africans rely on medicinal herbs for health care. Herbal medicine is becoming more popular in Nigeria and throughout West Africa due to its cost-effectiveness, accessibility, acceptance, and minimal toxicity (Sofowora, 1993). Globally, there is a reintroduction of traditional medicine due to its user-friendliness and the inherent adverse effects of contemporary drugs (Karunamoorthi et al., 2012). Scientific research has become increasingly interested in long-

standing traditional medical systems to examine the potential for producing novel phyto-therapeutic compounds (Karunamoorthi et al., 2012). Health problems are becoming more prevalent, and seemingly simple meals and food condiments may increase the effects of existing prescriptions. As a result of food scarcity, there is a growing quest for a scientific basis to employ plants and plant components as foods and pharmaceuticals, forcing a shift in that search to food and fruit waste. Furthermore, repurposing food and fruit waste as diets and therapies has the potential to reduce both environmental waste and associated public health problems (Chung et al., 1998; Pandey, & Rizvi, 2009). *C. papaya* is regarded as a nutraceutical fruit plant due to its many nutritional and health benefits (Santana et al., 2019).



Figure 1. The Leaves, fruit, and Stem of *Carica papaya*

The qualities of *C. papaya* fruit and other plant parts are well-known in traditional medical systems. Over the last few decades, significant progress has been made in the biological activity and therapeutic applications of papaya. Papaya has enormous therapeutic potential for a variety of diseases (Santana et al., 2019). The medicinal effects of the *C. papaya* plant's leaves, seeds, latex, and fruit have all been demonstrated. Latex can be found in abundance in papaya stems, leaves, and fruits (Aravind et al., 2013). Many active ingredients found in papaya leaves, including papain, flavonoids, cystatin chymopapain, tocopherol, ascorbic acid, cyanogenic glucosides, etc., have been shown to increase total antioxidant properties of the plant (Singh et al., 2020).

Carica papaya (Figure 1) grows in all tropical and subtropical countries across the world, with a lifespan of 5 to 10 years. *C. papaya* leaf is about 75 cm broad on long, hollow petioles, with the blades separated into 5 – 9 main segments (Morton, 1987). The fruit is usually long or spherical and contains about 1000 seeds (Siar et al., 2011). Natural chemicals found in plant leaf bark and twig tissues exhibit anti-tumor and pesticidal capabilities (Ogodo et al., 2015). Soil waterlogging usually leads to tree death within 3-4 days (Morton, 1987). Papaya thrives in well-aerated, well-drained, organic matter-rich soil with a pH of 5.5 to 6.7. Plants used for therapeutic purposes are frequently inexpensive, and they have a high concentration of pharmacologically active chemicals with high bacterial resistance (Basile et al., 1999). The edible component of *C. papaya* fruit contains Na, K, Ca, Mg, Fe, Cu, Zn, and Mn (Singh et al., 2020). The plant contains

carotenoids and several vitamins. Many active components of papaya leaves, including papain, chymopapain, cystatin, tocopherol, ascorbic acid, flavonoids, cyanogenic glucosides, and glucosinolates, have been shown to increase total antioxidant power in the blood and reduce lipid peroxidation (Mohamed, 2012; Santana et al., 2019; Lebaka et al., 2021). Pharmaceutical companies and academic organizations are always looking for new antibiotics to combat emerging and re-emerging resistant pathogenic organisms (National Institutes of Health USA, 2007; Fair, & Tor, 2014).

Thin-layer chromatography (TLC) is a chromatographic analytical method that separates mixtures, using an aluminum foil sheet impregnated with silica gel, or other suitable materials that function as stationary phase, and suitable solvent system that forms the mobile phase. Different analytes ascend the TLC plate, resulting in sample separation (Bunu et al., 2020). TLC can be used to track a reaction's progress, identify chemicals in a test sample, and determine compound purity (Jork et al., 1990). Phytochemical screening and TLC were used to determine the chemical composition of *C. papaya* leaves ethanolic extract.

MATERIALS AND METHOD

Plant Collection, and Extraction

Fresh leaves of *Carica papaya* were obtained in the wild, identified, and verified at the Department of Pharmacognosy and Phytochemistry, College of Pharmacy, Madonna University Nigeria. The leaves were properly washed, dried, and mashed with an electric blender to fine granules. About 250 g of the leaf fine granules were macerated in 600 ml of 100% Ethanol (analytical grade) for 72 hours before being filtered. The marc was then rewashed and filtered until all of the extractable components were removed. Both filtrates were mixed and concentrated using a rotary evaporator.

Phytochemical Analysis

The tests were conducted following previous procedures (Nwankwo et al., 2012; Bunu et al., 2023).

Alkaloids: approximately 20 ml of 3% sulphuric acid in 50% ethanol was added to 2g of extract and cooked in a boiling water bath for 10 minutes before being cooled and filtered, and then 2ml of the filtrate was poured into four test tubes labeled T1 - T4. A milky precipitate was detected after a few drops of Mayer's reagent were introduced to T1. A few drops of Dragendroff reagent were added to T2 filtrate, and a brick-red precipitate formed. A reddish-brown precipitate in the Wagner reagent indicates the presence of an alkaloid, but a yellow precipitate in Picric acid solution tests indicates the presence of an alkaloid.

Glycosides: 0.5g of powdered plant extract in a test tube was dissolved in 10 ml of 1% sulphuric acid and boiled for 15 mins, allowed to cool and neutralized with 20% KOH before adding 10 ml of the resultant mixture to an equivalent amount of Fehling solutions 1 and 2 and boiling for another 5 mins. A brick-red precipitate observed near the tube demonstrated the presence of glycosides.

Tannins: 0.5g of the powdered plant extract was dissolved in 20 ml of distilled water, and filtered. A few drops of ferric chloride were added to 3ml of filtrate, resulting in a greenish-black precipitate. A precipitate formed after a few drops of lead acetate solution were added to 3ml of filtrate. These reactions suggest the presence of tannins.

Flavonoids: 0.2g of extract of the powdered plant extract was dissolved in 10 ml of ethyl acetate, boiled for 3 minutes, and filtered. 4 mL of the filtrate was mixed with 1 mL of ammonia solution, the layers were allowed to separate, and a yellow coloration in the ammonia layer indicated the presence of flavonoids. In addition, 4ml of the filtrate was agitated with 1ml of 1% aluminum chloride solution before the layers separated. The formation of a yellow coloration in the aluminum chloride layer showed the presence of flavonoids in the sample.

Reducing Sugar: Fehling Solution Test: 5 mL mixture of the same proportion Fehling solutions 1 and 2 were added to 5 mL of extract and heated for 5 minutes in a water bath. There was no color change, indicating that decreasing sugar was not present.

Protein: 0.5g of extract was combined with 20 ml of distilled water before being filtered. The filtrate was employed in further research. In a test tube, two drops of Million's reagents were added to a small volume of filtrate. The formation of a white precipitate suggests the presence of proteins. 5 mL filtrate was heated with 3 drops of strong nitric acid (Xanthoproteic reagent), which produced a yellow hue that turned orange when diluted with NaOH, indicating the presence of protein.

Steroids and Terpenoids: 1g of the powdered plant extract was dissolved in 9 ml of ethanol, refluxed for 5 minutes, and filtered. 5 mL of hot distilled water was added after condensing the filtrate to 2.5 mL. After 1 hour, the mixture was filtered to remove waxy particles. The filtrate was extracted with 2.5 ml of chloroform with a separating funnel. 1 mL of conc. sulphuric acid was carefully added to 0.5 mL of chloroform extract in a test tube; a reddish-brown interface indicated the presence of steroids. Another 0.5 ml of chloroform extract was dried in a water bath before being heated for 10 minutes with 3 ml of conc. sulphuric acid; a grey coloration indicated the presence of terpenoids.

Saponins: 0.5 g of the powdered plant extract was dissolved in 20 ml of distilled water, boiled for 2 mins, and filtered. 5 mL of filtrate and 15 mL of distilled water were quickly mixed. Saponin presence was indicated by the development of a stable foam. About 2 drops of olive oil were added to the foamy solution and swirled thoroughly. The formation of an emulsion showed the presence of saponin.

Thin-Layer Chromatographic Analysis

Chromatogram preparation: Following the extraction method, the extract is placed in a beaker and dissolved in dichloromethane. For analysis, a TLC chamber was employed. A filter paper was cut and placed within the chamber to saturate the chamber with vapor, preventing the eluent from easily evaporating when it rose on the plate. After pouring a portion of the solvent into the

chamber, the chamber is tilted such that the solvent wets the filter paper. Close the chamber lid or place the watch glass on the beaker. Using a pencil, a straight line was drawn on the white surface 3cm from the bottom of the TLC plate and the locations where the samples were to be placed. The indicated regions should be 1cm away from the plate's edge and 1cm apart.

Spotting the TLC plate with the sample: A very small spot was placed on the line drawn on the TLC plate, allowed to dry thoroughly, and placed in the TLC chamber carefully, using forceps. The chamber lid was closed, the spotted sample was allowed to move close to solvent front, and the chromatogram was carefully removed from the chamber. Visible spots were determined and others were quantified using the UV lamp, all R_f values were carefully recorded.

RESULTS

Results obtained are presented in frequency tables and charts where necessary.

Table 1. Percentage Yield of the Extract

Variable	Quantity
Weight of dried leaves (W1)	250 g
Volume of ethanol	600 ml
Weight of dried extract (W2)	21.5 g
Percentage yield = $W2/W1 \times 100$	8.6 %

Phytochemical screening of *Carica papaya* leaves

The phytochemicals analysis of the ethanol extract of *Carica papaya* leaves was carried out and the test results were as seen in the table below.

Table 2: Phytochemical analysis of the ethanol extract of *Carica papaya* leaves

Test	Observation	Result	Inference
Alkaloids			
Mayer's reagent	Milky precipitate	+v	Alkaloid present
Wagner's reagent	Reddish brown precipitate	+v	Alkaloid present
Drangedoff's	Brick red precipitate	+v	Alkaloid present
Picric acid solution	Yellowish precipitate	+v	Alkaloid present
Carbohydrates			
Molisch's test	Purple ring formation	+v	Carbohydrate present
Glycoside			
Fehling's solution	A brick-red precipitate	+v	Glycoside present
Tannin			
Ferric chloride test	Greenish black precipitate	+	Tannin present

Lead acetate test	Formation of precipitate	+	Tannin present
Flavonoids			
Ammonia test	Yellow coloration	+	Flavonoid present
1% ammonium chloride solution	Yellow coloration	+	Flavonoid present
Reducing Sugar			
Fehling solution test	No color change	-	Reducing sugar absent
Protein			
Million's test	White precipitate	+	Protein present
Xanthoproteic test	A yellow color which changed to orange with the addition of alkali	+	Protein present
Steroids			
	Reddish brown interface	+	Steroid present
Terpenoids			
	A grey coloration	+	Terpenoids present
Saponins			
Frothing test	Froth formation	+	Saponin present
Emulsion test	Emulsion formed	+	Saponin present

Thin Layer Chromatography Analysis

$$R_f = \frac{\text{Distance moved by the sample}}{\text{Distance moved by the solvent front}}$$

Table 3. Chromatograms of the retention factor (R_f) value of *Carica papaya* leaves extract

Solvent System (ml)	Visually (Daylight)	Ultraviolet Light (245 nm)	Solvent System (ml)	Visually (Daylight)	Ultraviolet Light (245 nm)
Methanol (10)	0.55 (g)	0.55 (g)	n-hexane: ethyl acetate (7:3)	0.20 (g)	0.20 (g)
	0.65 (lg)	0.66 (g)		0.29 (y)	0.29 (y)
	0.72 (g)	0.72 (g)		-	0.40 (y)
	0.77 (y)	0.77 (y)		-	0.73 (y)
	0.82 (y)	0.82 (y)		-	0.88 (y)
	0.91 (g)	0.92 (g)			
Benzene: ethanol (9:1)	0.17 (g)	0.17 (g)	Petroleum ether: ethyl acetate (9:1)	0.25 (y)	0.25 (y)
	0.26 (g)	0.26 (g)		0.35 (y)	0.35 (y)
	0.66 (lg)	0.42 (b)		0.39 (g)	0.39 (g)
	0.82 (lg)	0.54 (b)		0.49 (g)	0.49 (g)
	0.92 (g)	0.66 (lg)		0.72 (g)	0.60 (lg)
	0.95 (g)	0.82 (lg)		0.81 (lg)	0.72 (g)

	-	0.91 (lg)		-	0.81 (lg)
	-	0.95 (g)			

Key: g = green, lg =light-green, y =yellow,

DISCUSSION

The plant extract was obtained at a low yield of 8.6% (Table 1). All of the reagents tested positive for alkaloids in the phytochemical screening, including Wagner's reagent, Mayer's reagent, Dragendoff's reagent, and picric acid solution. Protein and terpenoids were also found in the results. Tannin, saponin, flavonoids, and glycosides have also been found. During the phytochemical screening of these leaves, the absence of color change when the heated sample was reacted with Fehling's solution revealed the absence of reducing sugar. A basic phytochemical screening of *C. papaya* leaves revealed the existence of chemical components in ethanol extract via direct maceration with ethanol, revealing ethanol as a solvent capable of dissolving a wide range of molecules (Table 2). The existence of these phytochemicals could explain the reported pharmacological benefits of *C. papaya* leaves, such as anti-inflammatory, antibacterial, and anticancer characteristics (Singh et al., 2020). According to prior research (Sharma et al., 2022), these phytochemicals are also responsible for the usage of these leaves as nutritional supplements, prophylactics, and treatments for specific medical disorders.

The total number of spots obtained was 26. Using ethanol (10 mL) as the mobile phase, the chromatogram yielded 6 spots (3 green, 1 bright green, and 2 yellow) that could be visualized with ultraviolet light and the naked eye to show the presence of various chemicals. Chromatogram results employing petroleum ether: ethyl acetate (9:1) as mobile phase yielded 7 spots (2 light-green, 2 yellow, and 3 green spots) when visualized with ultraviolet light, and 5 spots when observed with naked eyes. This demonstrates the presence of several chemicals (Table 3).

When examined with the normal eye, chromatograms with the solvent system benzene: methanol (9:1) showed 6 spots (four green and two light-green spots), however when visualized with UV lamp, 8 spots were identified, including two brown, three light-green, and three green dots. For hexane: ethyl acetyl (7:3), UV lamp revealed 5 spots (four yellow and one green), and naked eyes revealed two spots (one green and one yellow spot). The distinct spots on the chromatogram and the corresponding R_f values from the various solvent mixtures used in the study demonstrate the presence of several phytochemicals in the plant. This variation in R_f values of phytochemicals provides important information about their polarity and aids in the selection of acceptable solvent systems for pure component separation. It also indicated the chemical elements that *C. papaya* leaves are likely to contain.

CONCLUSION

The leaf extract of *Carica papaya* was employed in this investigation because it is high in both primary and secondary metabolites, which may be responsible for the plant's purported pharmacological characteristics. Furthermore, Thin Layer Chromatographic profiling yielded an excellent result, pointing to the existence of many phytochemicals and potentially beneficial chemical constituents, which, when quantified, can serve as lead compounds in the design and development of valuable therapeutic agents.

REFERENCES

- Arvind G, Bhowmik D, Duraivel S, Harish G, (2013). Traditional and medicinal uses of *Carica papaya*, *Journal of Medical Carica Papaya*; 1(1):2320-3862.
- Basile A, Giordano S, Lopez-Saez J. A., Cobianchi R. C., (1999) *Phytochemistry* 52:1479-1482.
- Bunu J. Samuel, Ere Diepreye, and Wilson O. Diana (2020). Simple Thin-Layer Chromatographic and UV-Spectrophotometric Analysis of Promethazine and its N-Demethylation Metabolites from Biological Fluids. *International Journal of PharmTech Research*; 13, (04), 316-324, ISSN: 0974-4304.
- Bunu JS, D Alfred-Ugbenbo, ADC Owaba, B Okelekele (2023). Determination of phytochemicals and anti-bacterial properties evaluation of the leaves extracts of *Psidium guajava* (L) Myrtaceae. *European Journal of Pharmaceutical Research*, 3(3): 13 -16.
- Chavunduka EL, (1990). Christianity, African Religion and African Medicine in World Council of Churches.
- Chung KT, Wong TY, Wei CI, Huang YW, Lin Y (1998). Tannins and human health: a review. *Crit Rev Food Sci Nutr*; 38(6):421-64. doi: 10.1080/10408699891274273. PMID: 9759559.
- Fair, R. J., & Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st century. *Perspectives in medicinal chemistry*, 6, 25–64. <https://doi.org/10.4137/PMC.S14459>
- Jork, H., Funk, W., Fischer, W., Wimmer, H. (1990): Thin-Layer Chromatography: Reagents and Detection Methods, Volume 1a, VCH, Weinheim, ISBN 3-527-278834
- Karunamoorthi K, Tsehaye E, (2012). Ethnomedicinal knowledge, belief and self-reported practice of local inhabitants on traditional antimalarial plants and phytotherapy. *Journal of Ethnopharmacol.*141:143–150.
- Lebaka, V. R., Wee, Y. J., Ye, W., & Korivi, M. (2021). Nutritional Composition and Bioactive Compounds in Three Different Parts of Mango Fruit. *International journal of environmental research and public health*, 18(2), 741. <https://doi.org/10.3390/ijerph18020741>
- Mahomoodally MF, (2013). Traditional Medicine in Africa: An Appraisal of Ten Potent African Medicinal Plants Evidence-Based Complementary and Alternative Medicine, 10.1155/2013/617459.
- Mohamed Sadek K. (2012). Antioxidant and immunostimulant effect of *Carica papaya* Linn. Aqueous extract in acrylamide intoxicated rats. *Acta informatica medica : AIM : journal of the Society for Medical Informatics of Bosnia & Herzegovina : casopis Društva za medicinsku informatiku BiH*, 20(3), 180–185. <https://doi.org/10.5455/aim.2012.20.180-185>
- Morton JF (1987). Papaya. NewCROP, the New Crop Resource Online Program, Center for New Crops & Plant Products, Purdue University; from p. 336–346. In: Fruits of warm climates, JF Morton, Miami, FL. Retrieved 23 May 2015.
- Natako L, (2006). “Honouring the African Traditional Herbalist” In African Traditional Herbal Research Clinic Newsletter. Special Edition—HIV/AIDS.25 years;1(10).
- National Institutes of Health (US) (2007). Biological Sciences Curriculum Study. NIH Curriculum Supplement Series [Internet]. Bethesda (MD): National Institutes of Health (US); 2007. Understanding Emerging and Re-emerging Infectious Diseases. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK20370/>

- Nwankwo OL, Bunu JS, & Iloh ES, (2021). Standardization and antimalarial evaluation of the methanol and endophytic fungi extract isolated from the *Azadirachta indica* (Meliaceae) mice model. *Nigerian Journal of Clinical Pharmacy and Therapeutics*; 1(1):087-098.
- Ogodo, A. C., Ugbogu, O. C., Ugbogu, A. E., & Ezeonu, C. S. (2015). Production of mixed fruit (pawpaw, banana, and watermelon) wine using *Saccharomyces cerevisiae* isolated from palm wine. *SpringerPlus*, 4, 683. <https://doi.org/10.1186/s40064-015-1475-8>
- Pan, S. Y., Litscher, G., Gao, S. H., Zhou, S. F., Yu, Z. L., Chen, H. Q., Zhang, S. F., Tang, M. K., Sun, J. N., & Ko, K. M. (2014). Historical perspective of traditional indigenous medical practices: the current renaissance and conservation of herbal resources. *Evidence-based complementary and alternative medicine: eCAM*, 2014, 525340. <https://doi.org/10.1155/2014/525340>
- Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative medicine and cellular longevity*, 2(5), 270–278. <https://doi.org/10.4161/oxim.2.5.9498>
- Phua DH, Zosel A, Heard K, (2009). Dietary supplements and herbal medicine toxicities. *International Journal of Emerging Medicine*. 2(2):69-76.
- Santana, L. F., Inada, A. C., Espirito Santo, B. L. S. do, Filiú, W. F. O., Pott, A., Alves, F. M., ... Hiane, P. A. (2019). Nutraceutical Potential of *Carica papaya* in Metabolic Syndrome. *Nutrients*, 11(7), 1608. doi:10.3390/nu11071608
- Santana, L. F., Inada, A. C., Espirito Santo, B., Filiú, W., Pott, A., Alves, F. M., Guimarães, R., Freitas, K. C., & Hiane, P. A. (2019). Nutraceutical Potential of *Carica papaya* in Metabolic Syndrome. *Nutrients*, 11(7), 1608. <https://doi.org/10.3390/nu11071608>
- Sharma, A., Sharma, R., Sharma, M., Kumar, M., Barbhai, M. D., Lorenzo, J. M., Sharma, S., Samota, M. K., Atanassova, M., Caruso, G., Naushad, M., Radha, Chandran, D., Prakash, P., Hasan, M., Rais, N., Dey, A., Mahato, D. K., Dhupal, S., Singh, S., ... Mekhemar, M. (2022). *Carica papaya* L. Leaves: Deciphering Its Antioxidant Bioactives, Biological Activities, Innovative Products, and Safety Aspects. *Oxidative medicine and cellular longevity*, 2022, 2451733. <https://doi.org/10.1155/2022/2451733>
- Siar, S. V.; Beligan, G. A.; Sajise, A. J. C.; Villegas, V. N.; Drew, R. A. (2011). *Euphytica*, Volume 181, Number 2. *Euphytica*. SpringerLink. 181 (2): 159–168. doi:10.1007/s10681-011-0388-z. S2CID 40741527.
- Singh, S. P., Kumar, S., Mathan, S. V., Tomar, M. S., Singh, R. K., Verma, P. K., Kumar, A., Kumar, S., Singh, R. P., & Acharya, A. (2020). Therapeutic application of *Carica papaya* leaf extract in the management of human diseases. *Daru: journal of Faculty of Pharmacy, Tehran University of Medical Sciences*, 28(2), 735–744. <https://doi.org/10.1007/s40199-020-00348-7>
- Sofowora EA., (1993) *Medicinal Plants and Traditional Medicine in Africa*. 2nd ed. Ibadan, Nigeria: John Wiley and Sons Ltd, Spectrum Books. pp. 55-62
- W.H.O (2001). World Health Organization. *Legal Status of Traditional Medicine and Complementary/Alternative Medicine: Worldwide Review*.