INVITRO EVALUATION OF ANTIFUNGAL POTENTIALS OF METHANOLIC EXTRACTS OF THREE ORGANS OF *VITELLARIA PARADOXA* (SHEA PLANT)

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Abstract
Methanolic extracts of air dried leaf, stem bark and seed of Vitellaria paradoxa were analyzed for antifungal activities against five fungal species namely Trichophyton mentagrophyte, Aspergillus fumigatus, Epidermophyton flocossum, Trichophyton rubrum, and Microsporon audouinii. The plant organs were screened using the ditch method of media dilution technique. The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentrations (MFC) were determined using the broth dilution method. The activities of the extracts were compared with those of standard antibiotics. The extracts showed varying degree of activities against the test organisms between 0 - 100%. Extracts from the air-dried stem were more effective against the organisms than those from seed and leaf extracts. The methanolic extracts of the seed, stem bark and leaf showed fungistatic activity against the organisms at concentrations varying from 6.25mg/ml to 250mg/ml, but the leaf and seed extracts only showed fungicidal activity at concentrations as high as 250mg/ml against just two of the organisms, Trichophyton mentagrophyte and Microsporon audouinii, respectively. Phytochemical analysis of the extracts from the three organs of Vitellaria paradoxa revealed the presence of steroids, saponins, tannins, reducing sugars, flavonoids, general glycosides and anthraquinones. The medicinal potentials of the organs of Vitellaria paradoxa in the treatment of dermatitis or mycotic infection were discussed.

Keywords: Methanolic extracts, *Vitellaria paradoxa*, Dermatophytes, Phytochemicals, Media dilution technique.

Introduction
Finding healing powers in plants is an ancient idea and people on all continents have imbibed the infusions of hundreds if not thousands, of indigenous plants, dating back to prehistory. It is estimated that there are 250,000 to 500,000 species of plants on Earth of which a relatively small amount (1 to 10%) are used as foods and a larger proportion as medicines (Sheriff and Banik, 2006; Kubmarawa *et al.*, 2007).

Plants produce a vast array of structurally diverse secondary metabolites which serve as attractants for agents that mediate chemical defenses against pathogens which consists mostly of microorganisms (Wink, 2003). Over the years, studies have revealed the potentials of different medicinal plants.The medicinal values of these plants lie in their component phytochemicals which produce definite physiological actions on the human body (Afolabi *et al.*, 2007; Doughari and Manzara, 2008). Many drugs commonly used today are of herbal origin. Indeed, about 25% of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material (Doughari and Manzara, 2008). Some are made from plant extracts; while others are synthesized to mimic a natural plant compound. Salicylic acid, a precursor of aspirin, was originally derived from white willow bark and the meadowsweet plant. Cinchona bark is the source of malaria-fighting quinine. Vincristine, used to treat certain types of cancer, comes from periwinkle. The opium poppy yields morphine, codeine, and paregoric, a treatment for diarrhea. Even today, morphine-the most important alkaloid of the opium poppy-remains the standard against which new synthetic pain relievers are measured (Wink, 2003).

The Seed, Stem bark and Leaves of *Vitellaria paradoxa,* commonly known as Shea nut plant belonging to the Sapotaceae family was studied. It is a tree that grows naturally in the wild in 19 countries across the African Continent. In Nigeria it is locally abundant in the derived Savannah zones where it is considered economically useful as an essential oil producer, replacing the oil palm

as a source of edible oil in Northern Nigeria (Hall *et al.,* 1996). The Shea tree has a vast number of proven healing properties stemming from its chemical makeup of vitamin E, vitamin A, and cinnamic acid. It is also known to contain allantoin, a substance known to stimulate the growth of healthy tissue on ulcerous wounds (Hall *et al.*, 1996). It has been utilized by native Africans for more than a millennia (Steven *et al.,* 2003). Superficial skin infections are caused by a group of fungi known as Dermatophytes and causing infection known as *Tinea* or Ringworm (Sherif and Banik, 2006). Skin infections can also be caused by Species of *Aspergillus*.They are infections strictly confined to the outer epidermal (stratum corneum) tissues of the body and well adapted to breaking down keratin, the primary protein of epidermal tissue of vertebrates (skin, nail and hair) and are usually transmitted as a result of abraided skin and intimate contact (Steven *et al.*, 2003). Antifungal chemotherapy is in constant need of new and effective compounds due to the variable efficacy and adverse effects of drugs in current use (Sherif and Banik, 2006). Hence, characterization of additional sources especially from plants with better modes of action is of pivotal importance (Wink, 2003*).*

Materials and Methods

Source of Plant Materials: Samples of leaves, Seed and Stem of *Vitellaria paradoxa* were collected from Abakueji village of Ifelodun Local Government Area and University of Ilorin, permanent site campus, Ilorin, both in Kwara State, Nigeria in 2009. The samples were identified at the herbarium unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. Voucher specimen no UIH/04 was deposited at the herbarium.

Sample Preparation and Extraction Procedure: The leaf, seed, and stem of *Vitellaria paradoxa* were air dried for a period of about 2 weeks and ground into fine powder using a mechanical grinder. About 20g of the pulverized plant material (leaf, seed and stem) were packed in Soxhlet extractor using 250ml of methanol at a temperature of 67 $^{\circ}C$ for a period of 6 hours. The resulting filtrate was evaporated and concentrated using a rotary evaporator (model type 349/2, corning limited). The method of Akujobi *et al.* (2004) was adopted in the reconstitution of the extract. The various crude extracts were separately diluted with 30% dimethylsulphoxide (DMSO) to obtain varying concentrations of 250mg/ml, 200mg/ml, 150mg/ml, 100mg/ml and 50mg/ml. The initial concentration of 250mg/ml was obtained by weighing 1g each of crude extract from seed, leaf and stem bark into 4ml of 30% DMSO while the subsequent concentrations were achieved by greater dilutions with DMSO. These were refrigerated at 4^{oC} until required for the assay.

Selection of Fungal Isolates for Sensitivity Assay

The test fungi used in this study were *Trichophyton mentagrophyte, Aspergillus fumigatus, Epidermophyton flocossum, Trichophyton rubrum,* and *Microsporum audouinii*.

Determination of Antifungal Activities of Different Concentrations of Plant Extract

The effect of different concentrations of the methanolic extracts of three parts of *V. paradoxa* on the test isolates was investigated using the agar dilution technique. One milliliter of various concentrations of the plant extracts was separately incorporated into malt extract agar and allowed to solidify (Von-Etten,1973; Smith, 1977; Oloke *et al.*, 1988). Mycelial plugs of each test fungus measuring 5.0 mm in diameter was cut from the advancing margin of each fungal colony. A plug was placed at the center of each agar medium containing different concentrations of the plant extract (Ejechi and Akpomedaye, 1998). Control experiments contained the mycelial plug of the test fungus without the plant extracts. All experiments were carried out in replicates. plates were incubated at room temperature (28 + 2 ^{0} C) and diameter of growth measured after seven days for *Aspergillus* species and 18 days for *T. mentagrophytes, T. rubrum, M. audouinii* and *E. floccosum.* The results were expressed in % inhibition using the expression:

$$\%\text{inhibition} = \frac{y-z}{y} \times 100$$

where z= Diameter of growth after exposure to extract

y= Diameter of growth in malt extract agar without extract.

Determination of Minimum Inhibitory Concentration of Plant Extracts

Different concentrations of plant extract were added to malt extract test tubes and inoculated with water spore suspension of test fungi. The mixture was incubated and examined for growth. The least concentration of the plant extract that did not permit any visible growth of the inoculated organisms was regarded as MIC.

Determination of Minimum Fungicidal Concentration of Plant Extracts

Samples from tubes that inhibited growth in the MIC assay were re-inoculated unto freshly prepared agar plates. The lowest concentration of the extract that yielded no growth following subculturing on fresh agar plates was regarded as MFC in each case (Black, 1996).

Comparism of MFC of Extracts with those of Pure Antibiotics

This was carried out by comparing the MFC of the extracts with the MFC of pure antibiotics.

Phytochemical Screening of Leaf, Seed and Stem Bark Extract of V. *paradoxa.*

The method of Odebiyi and Sofowora (1978) with slight modifications was employed in the phytochemical screening of the extracts for the presence of the following biologically active compounds: Alkaloids, Tannins, general glycosides, Saponins, Steroids, Flavonoids, Anthraquinones and Reducing sugars.

Results

The effect of different concentrations of methanolic extracts of seed, leaf, and stem bark of *V. paradoxa* on the growth of five medically important test fungi was investigated and this indicated that all plant parts exhibited antifungal activity against the tested fungi. Generally the percentage inhibition of growth increased as the concentration of the extracts increased from 50mg/ml to 250mg/ml for most of the organisms (Figures 1 to 3). Assay of the seed extract against the test fungi (Figure 1) revealed that the extract did not inhibit *T. mentagrophytes* and *T. rubrum* at all at concentrations between 100mg/ml and 200mg/ml. The leaf extract (Figure 2) produced a better activity against the fungi than did the seed, however T. rubrum showed complete resistance to the extract at 50mg/ml and 250mg/ml concentrations. The result of the assay involving the stem bark exhibited the highest activities against all the tested fungi except for T. rubrum which showed no sensitivity at 100mg/ml concentration.

Plate I: Sensitivity plates of test organisms to methanolic extracts of leaf, seed and stem bark of *Vitellaria paradoxa*

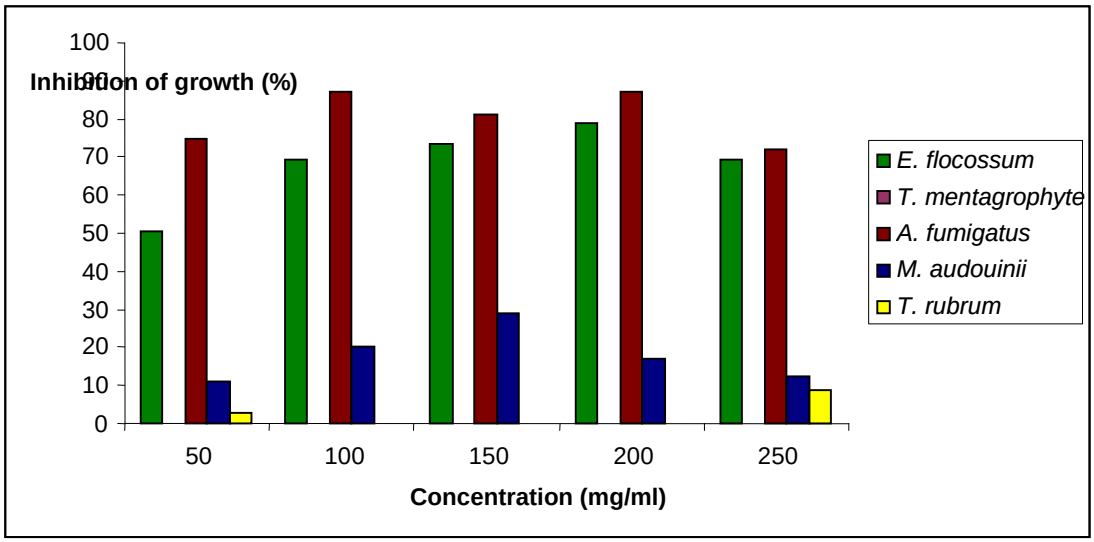

Figure 1: Sensitivity profile of test organisms to methanolic extract of seed of *Vitellaria paradoxa* at different concentrations

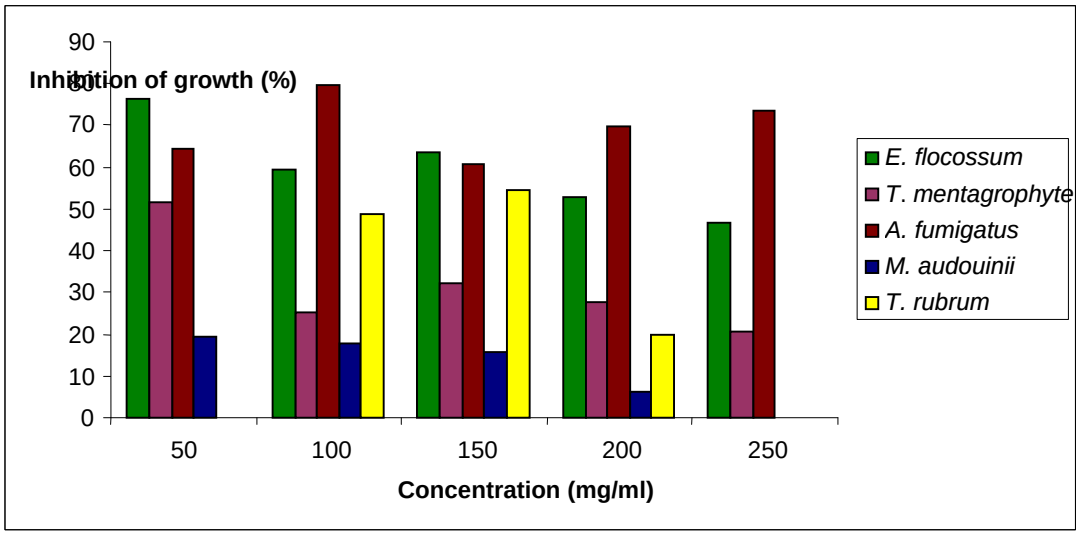

Figure 2: Sensitivity profile of test organisms to methanolic extract of leaf of *Vitellaria paradoxa* at different concentrations

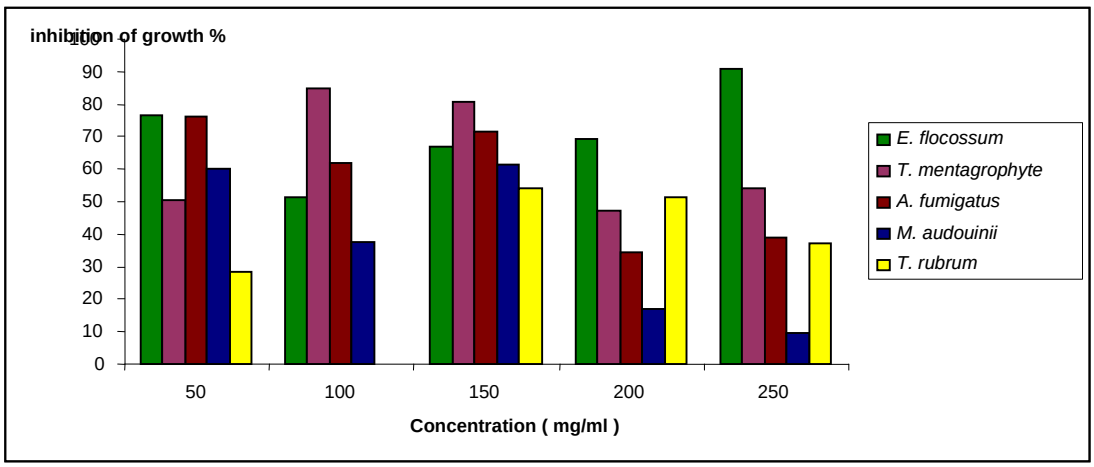

Figure 3: Sensitivity profile of test organisms to methanolic extract of stem bark of *Vitellaria paradoxa* at different concentrations

The result of the Minimum Inhibitory Concentrations of the plant extracts are presented in table 1. The MIC of extract from the stem of *V. paradoxa* were relatively lower than those for the leaf and seed (table 1). However, all plant parts showed inhibitory effect but at higher concentrations. This suggests that the stem back contains more bioactive constituents at higher concentrations. In the assesment of the minimum fungicidal concentration (MFC) of the extracts, the result indicate that MFCs were obtained at higher concentrations than for the MICs (Table 2) to completely exterminate the test organisms from growing.

Table 1: Minimum inhibitory concentration (MIC) of methanolic extracts of seed,leaf and stem bark of *Vitellaria paradoxa* on the test organisms

TEST ORGANISM	SEED EXTRACT		LEAF EXTRACT		STEM EXTRACT	
	MIC (mg/ml)	CONTROL	MIC (mg/ml)	CONTROL	MIC (mg/ml)	CONTROL
E. floccosum	150	NI	100	NI	50	NI
T. mentagrophyte	200	NI	150	NI	100	NI
A. fumigates	200	NI	200	NI	200	NI
T. rubrum	250	NI	200	NI	200	NI
M. audouinii	250	NI	250	NI	250	NI

NI = No Inhibition

Table 2: Minimum Fungicidal concentration (MFC) of methanolic extracts of seed, leaf and stem bark of *Vitellaria paradoxa* on the test organisms

TEST ORGANISM	SEED EXTRACT		LEAF EXTRACT		STEM EXTRACT	
	MFC (mg/ml)	CONTROL	MFC (mg/ml)	CONTROL	MFC (mg/ml)	CONTROL
E. floccusum	250	NF	200	NF	150	NF
T. mentagrophyte	250	NF	250	NF	250	NF
A. fumigates	NF	NF	NF	NF	250	NF
T. rubrum	NF	NF	NF	NF	250	NF
M. audouinii	250	NF	250	NF	200	NF

NF = No fungicidal activity

Generally the MFCs of the pure antibiotics were lower than those of the crude extracts when assayed against the test isolates. However the crude extracts of the stem bark proved more effective in the total elimination of *T. rubrum* at 250mg/ml while the pure antibiotics had no effect at all as seen in Table 3. The phytochemical analysis of the stem bark, leaf and seed of *V. paradoxa* are shown in Table 4. The stem bark revealed the presence of alkaloid, saponin, steroid, flavonoid, glycosides, anthraquinone and reducing sugars, thus possessing the highest number of bioactive ingredients. Alkaloids were generally absent in the leaf and seed extracts while the seed showed no presence of saponin.

Table 3: Comparism of MFC of methanolic extracts of *V. paradoxa* with selected standard antibiotics

	PLANT EXTRACTS (mg/ml)			ANTIBIOTICS (mg/ml)	
TEST ORGANISMS	STEM	SEED	LEAF	NYSTATIN	GRISEOFULVIN
E. floccusum	150	250	200	12.5	12.5
T .mentagrophyte	250	250	250	25	6.5
A. fumigatus	250	250	NF	6.5	NF
T. rubrum	250	NF	NF	NF	NF
M. audouinii	200	250	200	25	25

NF= No fungicidal activity

Table 4: Phytochemical analysis of aqueous extracts of stem bark, leaf and seed of *V. paradoxa*

Phytochemical	Stem bark	Leaf	Seed
Alkaloid	+	-	-
General glycosides	+	+	+
Saponins	+	+	-
Steroids	+	+	+
Flavonoids	+	+	+
Reducing sugars	+	+	+
Anthraquinones	+	+	+
Tannins	-	-	+

+ = present - = absent

Discussion

The findings in this work reveal that the stem bark, leaf and seed of the test plant possess a package of bioactive compounds that can be used in the treatment of infections caused by the test fungi used. The antimicrobial activity demonstrated by the methanolic extract is in agreement with previous studies that alcohols are noted to be reliable and consistent solvents for the extraction of antimicrobial substances from medicinal plants (Black, 1996). It was critically observed that the antifungal activity of the various extraction solvents increased as the concentration of the extracts was increased (Figure 1 to 3). This is in agreement with an investigation made by Banso (2005) and Kurosaki and Nishi (1983) who indicated that higher concentrations of aqueous extract of *Acalypha ceraceopunctata* increased its antifungal activity. The enhanced effect of the plant extracts with increasing concentration may be as a result of the presence of more crude materials of the plant in the higher concentrations which could have accounted for the release of more active chemical ingredients into smaller quantity of the extracting solvent and thus accounting for better activity than when less of the crude materials had been dissolved with more of the solvent thus rendering it a weaker solution and subsequently with reduced antifungal effect. It was also observed that the most effective extract was the stem bark while the seed proved the least effective. The reduced activity of the seed could be attributed to the presence of fat/oil in the seed which may have prevented the easy dissolution of active ingredients in the solvent. The enhanced activity of the stem bark might be due to the presence of more active phytochemicals which were present in the stem bark but absent in the leaf and seed which otherwise conferred more activity on the stem bark. This is in agreement with earlier reports that the stem bark usually produces better antimicrobial effects than other plant parts. A similar observation was made by Ndukwe *et al.* (2007) on the phytochemical and antimicrobial screening of crude extracts from the root, stem bark and leaves of *Vitellaria paradoxa*. In the assessment of the Minimum Inhibitory Concentration (MIC) the result indicates that the MICs of crude extracts from the stem bark (Table 1) of the plant was

relatively lower than those for the leaf and seed extracts. This observation suggests that the aqueous stem bark extracts may be more effective as an antifungal agent against the test fungi than the other plant parts. Antifungal agents with low activity against an organism have high MIC while those with high activity against such organisms have lower MIC (Emeruwa, 1982 ; El- Faraley *et al*., 1983).

The result of the Minimum Fungicidal Concentration (MFC) of the plant extracts indicate that higher concentrations than those of the MICs were required to obtain the minimum fungicidal concentrations (Table 2). Hence the antifungal substances contained in the extracts were fungistatic at high concentrations while becoming fungicidal at even higher concentrations of the plant extracts. This result correlates with the report of Acheampong *et al*. (1984) that large MIC or high concentrations of antimicrobial agents were needed to prevent the total growth of some microorganisms.

A comparative analysis of the MFC of extracts of *V. paradoxa* with pure antibiotics showed that generally the MFCs of the pure antibiotics (nystatin and griseofulvin) were lower than those of the crude plant extracts of *V. paradoxa* (Table 3). This implies that the antibiotics had higher antifungal activity than the crude extracts. Although in the two pure antibiotics did not possess an MFC concentration against T. rubrum at all while extract of the stem bark was able to exterminate the same fungus at a high concentration of 250mg/ml. Hence in order to enhance the antimicrobial activity of crude plant preparetions, it may be necessary to concentrate on the purification of the extracts in order to remove any impure substances which may be clogging to the active ingredients there by militating against the intended efficacy of the extracts. The phytochemical analysis of the crude aqueous extract of *V. paradoxa* plant parts revealed the presence of pharmacologically important phytoconstituents (Table 4).

Conclusion

In general this study indicates that the plant extracts are potent and can be used in the treatment of infections caused by the test fungi used. However further purification of the extracts could improve activity and may even compete with standard antibiotics. Also, *V. paradoxa* which is indigenous to most towns and villages in Nigeria could be utilized in the treatment of different forms of dermatitis prevalent among the rural communities were healthcare delivery is inadequate.

References

Acheampong, Y. B., Adimado, A. A. & Patel, K. S. (1984). Activity of some b-diketones. *International Journal of Pharmaceutical Society, 3, 207 – 209.*

Afolabi, C., Akinmoladun, E. O., Ibukun, I., Emmanuel, A., Obuotor, E. M. & Farombi, E. O. (2001). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. *Scientific Research and Essay, 2(5), 163-166.*

Akujobi, C. O., Anyauwa, B. N., Onyeze, G. O. C. & Ibekwe, V. I. (2004). Antibacterial activities of Preliminary phytochemical screening of few medical plants. *Journal of Applied Science, 7 (3), 4328-4338.*

Banso, A. (2005). *Studies on the antifungal properties of aqueous and alcholic extracts of three species of Acalypha.* Ph.D Thesis, University of Ilorin, Nigeria. Pp, 3 9-48.

Black, J. G. (1996). Antimicrobial therapy. In: *microbiology, principles and application 3rd edition.* New Jersey: Prentice Hall, upper Saddle. Pp. 356-358.

Doughari, J. K. & Manzara, S. (2008). Invitro antibacterial activity of crude leaf extracts of *Mangifera indica*. *African journal of Microbiology research, 2, 69-72.*

Ejechi, B.O. & Akpomedaye, D. E. (1998). The hurdle effect of ureolytic Proteus sp. Activity and *Trichoderma viride* culture filterate on growth and wood deteriorating activities of four fungi. *International Biodeterioration and Biodegradation, 41,153-155.*

EL- Faraley, Y. S., Cheatham, S. F. & Breelove, R. L. (1983). Antimicrobial neolignan of *Sassafras ranaiense root. Lloydia, 46 (4), 493-497.*

Emeruwa, A. L. (1982). Antimicrobial substancesfrom carcia papaya fruit extract*. Journal of Natural product, 45(2), 123-127.*

Hall, J. B., Aebischer, D. P., Tomlison, H. F., Osei-Amaning, E. & Hindle, J. R. (1996). *Vitellaria paradoxa*: *A monograph: school of agricultural and forest sciences.* University of Wales, Bangor, U.K.

Kubmarawa, D., Ajoku, G. A., Enwerem, N. M. & Okorie, D. A. (2007). Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. *African Journal of Biotechnolgy, 6(14), 1690-1693.*

Kurosaki, F. & Nishi, A. (1983). Isolation and antimicrobial activity of the phytoalexin-6-methyoxymellein from cultured carrot cells. *Phytochemistry, 22(3), 669-672.*

Ndukwe, I. G., Amupitan, J. O., Isah, Y. & Adegoke, K. S. (2007). Phytochemical and antimicrobial screening of the crude extracts from the root, stem bark and leaves of *Vitellaria paradoxa*. *African Journal of Biotechnology, 6,1905-1909.*

Oloke, J. K., Kolawole, D. O. & Erhun, W. O. (1988). The antifungal activities of certain components of *Aframonium meleguets* fruits*. Fitoterapia, 59(5), 384-388.*

Sherif, M. O. & Banik., G. R. (2006). Status and utilization of Medical plants in Rangamati of Bangladesh. *Journal of Agriculture and Biological Science*, *2,268-273.*

Smith, D. A. (1977). Observation on the fungi toxicity of phytoalexin, kevitone. *Phytochemistry 68: 81-87.*

Steven, M., Zeev, W. & Nissim, G. (2003). Phenolic constituents of Shea (*Vitellaria paradoxa)* Kernel. *Journal of Agriculture and Food Chemistry, 51, 6268-6273.*

Von-Etten, H.D (1973). Differential sensitivity of fungi to pistal and phaseolin. *Phytochemistry, 63, 1477-1482.*

Wink, M. (2003). Evolution of secondary metabolites from an ecological molecular phylogenic perspective. *Phytochemistry, 64,3-19.*