Full Length Research Paper

The use of *Psidium* guajava Linn. in treating wound, skin and soft tissue infections

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Accepted 18 May, 2009

The antibacterial potential of the crude leaves extracts of *Psidium* guajava Linn. against some bacteria associated with surgical wound, burns, skin and soft tissue infections were investigated under different conditions. Phytochemical screening of the crude leaves extracts revealed the presence of some bioactive compounds that have been associated with antimicrobial activities. Aqueous extracts was more potent in inhibiting the growth of pathogenic *Proteus mirabilis*, *Streptococcus pyogenes*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* than the organic extracts. The gram-negative bacteria were less susceptible to the effects of the crude drugs. The MIC and MBC values ranged between 6.25 - 50 mg/ml, depending on extracting solvent and the bacterium in question. Based on the diameters of zones of growth inhibitions, the crude extracts were more effective under acidic conditions and also at low temperatures. The plant can be used for the formulation of oral antibacterial drugs to manage surgical, skin and soft tissue infections.

Key words: Psidium guajava, antibacterial potential, bioactive compounds, surgical, soft tissue.

INTRODUCTION

Skin and soft tissue infections are infections involving the non-skelatal tissues. Most skin infections result from a break in the skin such as in surgery, decubitus ulcer, cuts, puncture, animal or insect bites, thorn and needle pricks or burns. When a hole is created on the skin, microorganisms, usually the opportunistic ones, invade the holes and multiply leading to a delay in the healing process and finally infectious condition. The spectrum of infection ranges from asymptomatic colonization to bacteraemia and death (Nester et al. 2004; Price, 1938). Colonization by opportunistic bacteria which begins immediately after birth is usually life long and may lead to infectious conditions whenever the skin is perforated. Some of the microorganisms frequently isolated in skin and wound infections includes Candida species, Staphylococci, Streptococci, Pseudomonads, Bacilli and Escherichia coli amongst others. These bacteria have greater resistance and virulent capabilities including the formation of biofilms on colonized surfaces (Price, 1938; Grenet et al., 2004). Eradication of these pathogens has been shown to result in rapid wound healing. Complications from burns, surgical wounds, skin and soft tissues arise from the colonization of such sites by some bacteria and fungi (Pfaller et al., 2001; Poikonen et al., 2003;

Muhammad and Muhammad, 2005). These complications can be avoided by proper sanitation and good hygienic practices. Some antibiotic therapies are usually initiated at health facilities, but in most cases, patients simply stay at home and nurse themselves back to health using local herbs and plants. The therapeutic efficacies of many of these indigenous plants have been described by practitioners of traditional medicine and other knowledgeable people in the rural areas (Muhammad and Muhammad, 2005; Mohanasundari et al., 2007). Some plants have been employed in the traditional herbal medicine for only treatment purposes while others as medicines and foods, spices and condiments in foods for hundreds of years in many countries of the world and were found to have the advantage of little or no adverse effects (Steward and Besswick, 1989; Sofowora, 1982).

Psidium guajava Linn. is one of such medicinal plants belonging to the family Myrtaceae that is also used as a source of food. It is a native of central America but is now widely cultivated throughout the tropics. It is one of the most gregarious of fruit trees and is widely known by its common English name or its equivalent in other languages (it is called guava in Hausa and Fulfulde, goyave in French, guava in Malay, gurfa in Yoruba and Gwaibwa

in Ibo). It is a genus of about 100 species of tropical shrubs and small trees. Thriving in all types of soils, the guava is widely distributed and the fruits enrich the diets of millions of people in the tropics of the world. It is a small tree about 10 m high, with spreading branches that thrives on all kinds of soils. Guava, also known as the poor mans apple of the tropics, has a long history of traditional use, a good proportion of which have been validated by scientific research (Burkil, 1994). Some of the ethnomedicinal uses includes the crushing of the leaves and the application of the liquids coming out from them on wounds, cuts, ulcers, boils, skin and soft tissue infectious site, rheumatic places (Bala, 2006) and the chewing of the leaves to relieve toothache, oral ulcers, inflamed gums, throat, chest pains, treatment of leucorrhea, diarrhea, dysentery, convulsions and epilepsy, as well as the use of the decoctions and infusions as a douche for vaginal discharges and to tighten and tone of vaginal walls after childbirth (Burkil, 1994). In some cultures, a decoction of the leaves is drunk to regulate menstrual periods and expel the placenta after child birth (Lozoya et al., 2002). Its anti-amoebic and antimalarial effects have also been documented (Morton, 1987, Tona et al., 1998).

The phytochemical components of the guava plant have been established in previous studies. Guava was reported to be rich in tannins, phenols, triterpenes, lectins, quercetins, leucocyanidins, sequiterpenes hydro-carbons, caryophyllenes, sterols, gallic acid, guavins A, C and D, carotenoids, vitamins, fibres and fatty acids (Morgan, 1987; Akinpelu and Onakoya, 2006; Kamath et al., 2008). Several in-vitro studies have shown significant antimicrobial activities against Staphylococcus, Shigella, Salmonella, Bacillus, E. coli, Clostridium, Pseudomonas and Candida spp. (Akinpelu and Onakoya, 2006; Mbuh et al., 2008). Since rural people have developed techniques in treating their cuts, wounds, burns, skin and soft tissues by application of a paste made from the fresh or dried leaves of the guava and there are no published reports to support that, it will be interesting to investigate the usefulness of the plant in treating these infections. The present study is aimed at evaluating the antimicrobial potential of crude leaf extracts of Psidium guajava Linn. against pathogenic bacteria associated with wound, skin and soft tissue infections. The effects of pH and temperature on the efficacy of the crude extracts have also been investigated to simulate the acidic conditions found in the stomach and the gastrointestinal tract.

MATERIALS AND METHODS

Collection of plant materials

Young fresh leaves of *P. guajava* was collected from Sangere, a sprawling settlement just opposite the main gate of the federal university of technology, Yola, Nigeria. The leaves were identified in biological science department of federal university of technology, Yola.

Preparation of plant material

The fresh leaves were washed with tap water, chopped into smaller pieces with a knife and then kept in the shade for 14 days to dry and then crushed using pestle and mortar and further reduced to powder using electric (Kenwood) blender and then stored in airtight closed bottles until required.

Extraction procedure

10 g of the powdered sample of the plant was soaked in 100 ml of sterile distilled water in a 250 ml conical flask at room temperature (32 - 35°C) with shaking after every 4 for 24 h. The extract was filtered using muslin cloth and then Whatman no.1 filter paper. The filtrates was then evaporated to dryness in a rotary evaporator maintained at 45°C to remove residual solvents and then stored in screw capped bottles for further use. The same experimental procedures were used for the other solvents, namely, acetone and methanol.

Microorganisms

The test bacteria were *Streptococcus pyogenes, Proteus mirabilis, Pseudomonas aeruginosa and Staphylococcus aureus* and *E-coli* isolated from clinical specimens obtained from patients diagnosed with surgical wound, skin and soft tissues infections at the 750-bed specialist hospital, Yola, Nigeria. Collection, transportation of specimens and isolation of bacteria were performed following standard microbiological procedures as described by Cheesbrough (2006). Identification of bacteria was done using the methods described by Cowan and Steel (1974).

Innoculum standardization of culture was done according to the methods described by Baker and Thomsberg (1983) and the nation-nal committee for clinical standards (NCCLS), (now known as clinical laboratory standards institute (2006). Briefly, some 18 h culture of test bacteria was suspended into sterile universal bottles containing Mueller Hinton broth. Normal saline was added gradually to it, so as to compare the turbidity with 0.5 MacFarland standard which gives approximately 108 cells/ml.

Phytochemical analysis of the leave extract

The methods described by Odebiyi and Sofowora (1978) and Fennell et al., (2004) was used to screen for the presence of the phytoconstituents. The dried extracts were first reconstituted in the respective solvents used for their extraction and then tested by standard phytochemical methods for the presence of some bioactive compounds, namely, tannin, balsam, alkaloid, carbohydrates, phenol, saponin, flavonoids and cardiac glycosides.

Screening for antibacterial activity

The antibacterial test was performed using the agar well diffusion method (Akoma and Olawepo, 2002). 1 ml of 18 h culture of the test bacteria previously adjusted to 0.5 MacFarland standards corresponding to approximately 1.0 x 10^8 cfu/ml was pippeted into a sterile plate and then 19 ml of molten Mueller Hinton agar at $45^{\circ}\mathrm{C}$ added and the plate shaken gently so as to mix the contents. The molten agar and the test organisms were allowed to solidify on a flat bench for 30 min. A sterile cork borer 6 mm diameter was used to drill holes 4 mm deep. 0.5 ml of the plant extract at a concentration of 25 mg/ml was placed in each of the three holes. Then 0.5 ml of solution of the pure solvent was pippeted into the fourth hole to serve as negative control and 0.5 ml of 10 µg/ml gentamycin was

used as positive control. The plates were incubated at 37°C for 24 h. Antibacterial activity was determined by measuring zone of growth inhibition and mean of triplicate results taken.

Effects of pH and temperature on the activity of the crude extracts

This was carried out as previously described (El-Mahmood et al., 2008). Briefly, 25 mg of dried powdered sample was dissolved in 1 ml of solvent. Then few drops of either 1N HCl was added dropwise on to the extracts until a pH 2 was reached. Similarly, few drops of 1 M NaOH was added drop-wise in to the second test tube containing 25 mg/ml of the extract in a second test tube, until a pH 10 was reached. The treated test tubes were left to stand for 1 h and the neutralized to pH 7 by adding either acid or base as the case may be. A third test tube did not contain either acid or base and served as positive control. 0.5 ml of the treated and untreated extracts were then introduced into 4 mm agar wells previously bored on the surfaces of agar plates, seeded with an 18 h old cu-Iture of bacteria and incubated for 24 h. Solutions of the pure solvents served as negative controls. Antibacterial activity of both the treated and untreated cultures was determined as previously described.

For the determination of the effect of temperature on activity, the extracts at a concentration of 25~mg/ml were treated at different temperatures of between 10 - 100°C in different sets of test tubes for 1 h and then brought back to room temperature. Another test tube was left at room temperature and served as positive control. Then 0.5~ml of the treated and untreated extracts were introduced into wells bored on the surfaces of agar plates that were previously seeded with the test bacteria adjusted to $1.0~\text{x}~10^8~\text{cfu/ml}$. Solutions of the pure solvents served as negative controls.

Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the crude extracts

The MIC of the extract were determined using doubling broth dilution method of Sahm and Washington (1990). The powdered sample was reconstituted by dissolving 100 mg of the powder in 1 ml of Mueller Hinton broth, then 1ml of the extract at this concentration was transferred to another 1 ml of broth in another test tube and this continued untill a 7^{th} test tube was reached. The 8^{th} test tube did not contain any extract and only sterile Mueller Hinton broth served as control. Then 1 ml of an 18 h culture with a cell density of 1.0×10^{-8} cfu/ml was inoculated in each test tube and the contents thoroughly mixed on a votex mixer. The test tubes were then incubated at 37^{0} C for 24 h. The tube with lowest dilution with no detectable growth in form of turbidity was considered the MIC (El-Mahmood et al., 2008).

For the MBC determination, 100 μ l of the broth culture were taken from the MIC test tube that showed no growth by visual inspections and inoculated on to the surfaces of a solidified Mueller Hinton agar contained in 90 mm petri dishes.

Statistical analysis

The results are presented as mean \pm SD at P > 0.05 level of significance.

RESULTS AND DISCUSSION

Phytochemical and antimicrobial screening methods could provide the needed preliminary observations nece-

ssary to select among crude extracts, those with potentially useful properties for further chemical and pharmacological investigations (Aliero and Afolahan, 2006). Elucidation of the chemical structures of these compounds can lead to the synthesis of more potent drugs with minimal toxicity. Many of the pharmaceuticals presently prescribed in hospitals have a long history of use as herbal remedies including quinine, belladonna, digitalis, emetine, strychnine and aspirin. The results of the phytochemical screening of the leaves of Psidium guajava revealed the presence of tannin, saponin, balsam, alkaloids, phenols, saponin, cardiac glycosides and carbohydrates. Umeh et al. (2005) reported that many of the phytoconstituents with antibacterial proper-ties in plants are preferably concentrated in leaves. The accumulation of these compounds in plant cells have attracted the attention of scientists worldwide and have been the subject of intense investigations. These bio-active constituents which serve as protective substances against infections by bacteria, fungi, viruses and pest, also have some medicinal uses. Phytochemicals exert their antimicrobial activities through different mechanisms. Tannins for example, have been reported to irreversibly form complexes with proline-rich proteins, resulting in the inhibition of cell protein synthesis. Tannins also react with proteins to produce the typical tanning effect and this is important in the treatment of inflamed or ulcerated tissues, burns, wounds, pneumonia and dysentery (Mota et al., 1985). Plant parts that contain tannins are astringent in nature and have important roles as stable and potent antioxidants (Dharmanda, 2003). Phenols, terpinoids, saponins and flavonoids found in the leaves of guava have been observed to exert anti-bacterial properties (Marjorie, 1999; Ogbonnia et al., 2008) and together with the alkaloids and tannins in a synergistic manner, are responsible for the inhibition of growth observed in this study. Much of guava's therapeutic activity is attributed to tannins, phenols and flavonoids, in particular, quercetin (Morton, 1987; Scalbert, 1991). Also, lectin chemicals in guava were shown to bind to E. coli, preventing its adhesion to the intestinal wall and thus preventing infections (Lutterodt, 1989).

The antibacterial activity of the crude extracts of the leaves of *P. guajava* was evaluated by measuring the sizes of zones of growth inhibition produced by the extracts against the test bacteria as detailed in Table 1. These inhibitions of growth of bacteria were as a result of the presence of the phytoconstituents identified in the crude extracts. The susceptibility of the bacteria to the extract on the basis of growth inhibition zone diameters, varied, according to microorganism, but generally, the largest inhibition zone diameters were recorded with the gram-negative bacteria and this is in agreement with the reports of El-Mahmood (2009). Based on the zones of growth inhibition sizes, the aqueous extracts was more potent, producing zone sizes of 16 mm against *Proteus mirabilis*, 18 mm *against S.* pyogenes, 20 mm against *S.*

Table 1. Phytochemical constituents of *P. guajava* leaf extract.

Phytoconstituents		Solvents	3
	Methanol	Acetone	Distilled water
Tannin	+	+	+
Saponin	+	+	+
Balsam	+	+	+
Flavonoid	+	+	+
Alkaloid	+	+	+
Cardiac glycosides	+	-	+
Phenol	+	-	+
Carbohydrate	+	+	+

Key: + positive; - negative

Table 2. Antibacterial activities of extract of Psidium guajava Zone of inhibition diameter (mm).

Organism	Acetone	Methanol	Distilled water	Control (10 gentamycin)
P. mirabilis	14 ± 0.82	15 ± 0.82	16 ± 0.82	28 ± 0.82
P. aeruginosa	$8n \pm 0.82$	10 ± 0.82	11 ± 0.82	17 ± 0.82
S. pyogenes	16 ± 0.82	16 ± 0.82	18 ± 0.82	27 ± 0.82
E. coli	12 ± 0.82	10 ± 0.82	13 ± 0.82	24 ± 0.82
S. aureus	18 ± 0.82	16 ± 0.82	20 ± 0.82	30 ± 0.82

Values are means of 3 determinations ± D.

aureus, 13 mm against E. coli and 11 mm against P. aeruginosa. The patterns of growth inhibition were similar for the methanol and acetone extracts. The methanol extract was more effective than acetone and both were inferior to water extract, similar to the observations of El-Mahmood et al. (2008) but contrary to the reports of Mbuh et al. (2008). The limited spectrum of activity shown by the acetone and methanol extracts compared with the water extracts are difficult to explain since all the extracts contained the metabolites, though in different proportions. Perhaps the active principles are more soluble in water than the organic solvents. This can only be resolved when the bioactive compounds have been isolated and the molar activity of the purified form determined. Mbuh et al. (2008) investigated the antibacterial activity of the leaves extract of P. guajava and reported that the crude extracts inhibited the growth of S. aureus, S. typhi, E. coli, B. subtilis, Shigella spp., P. mirabilis and K. pneumoniae and that the methanol extract was more potent. In a similar study, Abdelrahim (2002) reported that the methanol extract was more effective in inhibiting the growth of B. subtilis, S. aureus, E. coli and P. aeruginosa. Okwori et al. (2008) had documented the susceptibility of P. aeruginosa, S. aureus and S. typhi to plant extracts, while El-Mahmood (2009) reported the susceptibility of nosocomial E. coli, S. aureus, S. pneumoniae and P, aeruginosa to crude extracts of garlic. Pathogenic bacteria have evolved numerous defence mechanisms against antimicrobial agents including plant based products. Gram-negative bacteria are generally more resistant to antibacterial agents, because apart from their complex cell wall structures, some of them like P. aeruginosa maintain antibiotic resistance plasmids and are able to transfer these genes by means of bacterial processes of conjugation and transduction to other bacteria in their immediate vicinity (Adebayo et al., 1989; Akinpelu and Onakoya, 2006; Mbuh et al., 2008; Lal et al., 2008). The slightly higher resistance of E. coli and P. aeruginosa may be due to the inability of the extracts to penetrate the cell walls and get to the susceptible sites in the 2 bacteria (Lal et al., 2008). The presence of thick murine layer in the cell wall prevents the entry of environmental substances including antibiotics and plant based biocides(Khandhasamy et al., 2008). The ability to inhibit the growth of S. aureus, Proteus mirabilis S. pyogenes, E. coli and P. aeruginosa as shown in Table 2 is an indication of broad spectrum activity of the extracts.

Traditionally *P. guajava* leaves are usually chewed or soaked in cold water before application on skin surfaces to treat cuts, burns, boils and other skin infections. In some cultures, the *P. guajava* leaves are boiled before being taking orally as remedy to various ailments and infectious conditions. The effects of temperature on the activity of the crude extracts are shown in Table 3. The extracts were more effective at lower temperatures. The activity decreased as temperature was raised to boiling. Treatment of extract at high temperature could decrease antimicrobial activity in some ways, it could destroy volatile

Table 3. Effect of temperature on P. guajava on leaf crude extract zone of inhibition diameter (mm).

	Acetone		Metl	nanol	Distilled water	
Organism	10 ⁰ C	100 ⁰ C	10 ⁰ C	100 ⁰ C	10 ⁰ C	100°C
P. mirabilis	16 ± 0.82	12 ± 0.82	15 ± 0.82	10 ± 0.82	17 ± 0.82	12 ± 0.82
P. aeruginosa	9 ± 0.82	7 ± 0.82	8 ± 0.82	7 ± 0.82	10 ± 0.82	8 ± 0.82
S. pyogenes	15 ± 0.82	13 ± 0.82	15 ± 0.82	12 ± 0.82	18 ± 0.82	15 ± 0.82
E. coli	13 ± 0.82	10 ± 0.82	15 ± 0.82	10 ± 0.82	15 ± 0.82	11 ± 0.82
S. aureus	16 ± 0.82	13 ± 0.82	19 ± 0.82	13 ± 0.82	20 ± 0.82	16 ± 0.82

Values are means of 3 determinations ± SD.

Table 4. Effect of pH of *P. guajava* leaf extract (zone of inhibition diameter) mm.

	Methanol		Acetone		Distilled water		Control	
Organism	pH2	pH10	pH2	pH10	pH2	pH10	pH2	pH10
P. mirabilis	19 ± 0.82	15±0.82	20 ± 0.82	18 ± 0.82	19 ± 0.82	16 ± 0.82	16 ± 0.82	14 ± 0.82
P. aeruginosa	9 ± 0.82	6 ± 0.82	8 ± 0.82	6 ± 0.82	10 ± 0.82	7 ± 0.82	17 ± 0.82	15 ± 0.82
S. pyogenes	15 ± 0.82	13 ± 0.82	27 ± 0.82	23 ± 0.82	20 ± 0.82	16 ± 0.82	16 ± 0.82	14 ± 0.82
E. coli	30 ± 0.82	26 ± 0.82	28 ± 0.82	2 ± 0.824	16 ± 0.82	13 ± 0.82	18 ± 0.82	14 ± 0.82
S. aureus	19 ± 0.82	16 ± 0.82	18 ± 0.82	14 ± 0.82	19 ± 0.82	15 ± 0.82	15 ± 0.82	14 ± 0.82

Values are means of three determinations ± SD.

compounds such as enzymes and vitamins. The results in this study do not support boiling of the plant parts as is used in some cultural settings. Also, in some cultures, the herbalists usually add some potash (called Kanwa in the Hausa language) to improve in the solubility and on the potency of the decoctions and infusions. In this study, the efficacy of the extracts were more under acidic conditions as shown in Table 4, which again support the techniques used traditionally. Alkali treatment of the crude extracts has a negative effect. The efficacy of the extracts under low pH and also low temperature is an indication that it can withstand the conditions in the stomach and gastrointestinal tract and therefore can be formulated to be taken orally. Throughout this study, the solutions of the pure solvents used as negative controls did not show any significant activity in form of growth inhibition against the test bacteria. However the standard antibiotic gentamicin. though at lower concentration than the crude extracts, produced larger zones of growth inhibition than the crude extracts, meaning that gentamicin was more effective than the extracts as shown in Tabl0e1. Similar observations have been reported by by other scholars (De and Ifeoma, 2002; Kubmarawa et al., 2002; El-Mahmood and Amey, 2007). Generally, antibiotics obtained from microorganisms and or the synthetic processes are more effective at lower doses that plant based products. The scientific literature is full of reports of bioactive compounds isolated from plants having antimicrobial properties, but none of these plant based chemicals have been successfully exploited for clinical uses as antibiotics (Gibbons, 2004).

The MIC is a helpful parameter used to assess bacteriostatic activity of antibacterial agents while the MBC is used to detect bactericidal activity under similar conditions. The MBC values are generally more reliable than the MIC values which are obtained using turbidity as an index (Junaid et al., 2006). This study showed that E. coli and P. aeruginosa have higher MIC and MBC values which translated that a higher concentration of the extracts are required to inhibit their growth and this agrees with the reports of Ogbonnia et al. (2008). Hassain (2002) had suggested that the quantity of active ingredients of plant origin required to cause inhibition of growth may not matter since medicinal plants have been reported to have little or no side effects. The MIC values can either be the same or lower than the MBC values (Croshaw, 1983). The MIC values ranged from 6.25 - 25 mg/ml while the MBC values were also low and ranged from 6.25 - 50 mg/ml. This low MIC and MBC values shown in Table 5 are indications of potency of the plant constituents against the pathogens and attests to its use in traditional medicine to treat various infectious conditions including those caused by such recalcitrant bacteria as those used in this study. This relatively low values reveals the effecttiveness of the extracts and this is very important for people who depend on this plant for their health care needs. Discussion with a large number of traditional medicine practitioners in the north-eastern parts of Nigeria revealed that they normally press the juice from the fresh leaves on the wound or infected skin or use the cold decoctions to treat burns, boils, cuts and other skin infections. Hence an acceptable and effective dosage can be

S. pyogenes

E. coli

S. aureus

ude extract of 7.	Methanol	l (mg/ml)	Acetone (mg/ml)		Distilled water (mg/ml)	
Organism	MIC	MBC	MIC	MBC	MIC	MBC
P. mirabilis	12.5 ± 0.82	25 ± 0.82	25 ± 0.82	50 ± 0.82	6.25 ± 0.82	6.25 ± 0.82
P. aeruginosa	25 ± 0.82	50 ± 0.82	25 ± 0.82	50 ± 0.82	25 ± 0.82	25 ± 0.82

 12.5 ± 0.82

 25 ± 0.82

 6.23 ± 0.82

 25 ± 0.82

 50 ± 0.82

12.5 ± 0.82

Table 5. Determination of inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of leaf crude extract of *P. guajava*.

Values are means of 3 determinations ± SD.

 12.5 ± 0.82

 25 ± 0.82

 6.25 ± 0.82

 25 ± 0.82

 25 ± 0.82

 12.5 ± 0.82

obtained by the traditional healers for the control and eradication of the bacterial pathogens by pressing the juices from either the chewed or crushed leaves on the infectious sites. This in is agreement with the results obtained in this study since the extracts were found to inhibit the most common bacteria that cause surgical wound, skin and soft tissue infections.

Conclusion

The result obtained for this study shows that *P. guajava* leave extracts have antimicrobial activity against bacteria that commonly cause surgical wound, skin other soft tissue infections. This study has provided a scientific basis on the use of crude extracts of guava in herbal medicine. Its full potential in the pharmaceutical Indus-tries is however dependent on the full identification, purification and characterization of the biologically active components in the plant.

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 12.5 ± 0.82

12.5 ± 0.82

 6.25 ± 0.82

 6.25 ± 0.82

 12.5 ± 0.82

 6.23 ± 0.82

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