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Research Article

Effects of solvents on phytochemicals and antimicrobial activity of leaf extract from *Vigna unguiculata*

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Abstract

Cowpea (*Vigna unguiculata* (L.) Walp), an indigenous African legume crop, is used to treat epilepsy, bilharzia, chest pains and constipation. The leaf extract of *V. unguiculata* is an important source of polyphenolics and other phytochemicals with different functional effects like antimicrobial and antioxidant properties. This study strongly lay emphases on the effects of solvents on phytochemical and microbial properties of leaf extracts of *V. unguiculata*. The solvents like Ethanol, Ethyl ether, n-hexane and aqueous were used for the crude extracts for determination of the phytochemicals and microbial potentials of the leaf of *V. unguiculata* extract. The results of phytochemical screening of *V. unguiculata* leaf extract has shown the potency of the plant for antimicrobial activity. The antimicrobial activity of the different solvents shows greater activity against different bacterial strains which was performed by agar well diffusion method. Ethanol extract of Vigna leaf shows greater activity against bacterial strains compared to the other three solvents, n-Hexane extract shows least activity but shows greatest activity against E. coli as compared to ethanol, Ethyl acetate and aqueous extracts. Ethanol extracts of Vigna leaf shows greater activity against H. pyroli almost the same strength with standard drug (Amoxicilin 200 µg). From the results obtained it shows Ethanol extracts of *V. unguiculata* leaf extract gives better potency against bacterial activity.

Keywords: *V. unguiculata*; Phytochemical; Antimicrobial activity; Microorganisms; Extracts.

Introduction

Ancient texts of India and China contain exhaustive depictions of the use of a variety of plant-derived medications [1]. In fact, plants remain the main source of medicines for a large proportion of the world's population, particularly in the third world countries [2]. The use of plants remains undisputed despite the advent of the Pharmaceutical and natural product Chemistry during the early twentieth century, which brought with it the ability to synthesize an enormous variety of drug molecules and thereby allowed the treatment of previously incurable and/or life-threatening diseases [3].

Drugs from natural sources are less toxic and many are very effective. About half of the world medicinal compounds are derived from plants. Medicinal products from plants are generally more important in developing countries than in developed and industrialized world as 75–90 % of the world's rural populations rely comfortably on herbal medicines [4]. Africa is a rich ground for many very useful plants from time immemorial but they are largely undocumented, the knowledge are always kept secret within a family or clan and passed from one person to the other leading to a large information being lost from one generation to the other through inappropriate folklore passage of information or sudden deaths of the people or person(s) in the current custody of the knowledge [5].

Vigna unguiculata (cowpea) originated in Africa, where a large genetic diversity of wild types occurs throughout the continent, southern Africa being richest. The greatest genetic diversity of cultivated cowpea is found in West Africa, in the savanna region of Burkina Faso, Ghana, Togo, Benin, Niger, Nigeria and Cameroon. Cowpea is the most important pulse crop in the savanna regions of West and Central Africa, where it is also an important vegetable and a valuable source of fodder. In East and southern Africa it is also important both as a

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vegetable and a pulse. Only in humid Central Africa is it less prominent. Cowpea serves as a cheap source of plant protein. Various medicinal uses of cowpea have been reported by scholars that the leaves and seeds are applied as a poultice to treat swellings and skin infections, leaves are chewed to treat tooth ailments, powdered carbonized seeds are applied on insect stings, the root is used as an antidote for snakebites and to treat epilepsy, chest pain, constipation and dysmenorrhoea, and unspecified plant parts are used as a sedative in tachycardia and against various pains. Authors in [6] reported that an infusion of the seed of *Vigna unguiculata* can be taken orally to treat amenorrhoea whilst powdered roots eaten with porridge are believed to treat painful menstruation, epilepsy and chest pain by the indigenous people of South Africa. Leaves are applied on burns and can be used as a snuff to treat headaches [7].

Cowpea has also been identified as a plant that traditional healers use to treat urinary *shistomiasis* (bilharzia) in Zimbabwe [8]. Cowpea seeds cooked with the roots of *Lannea edulis*(Sond.) Engl. [6], *Euclea divinorum* or *Terminalia sericea* are used to treat blood in the urine and bilharzias by South Africans [9]. Some antibiotics in use today like Penicilins, Fluoroquinolones, Cephalosporins,Imidazoles etc, their use is being threatened or limited by the development of resistance by microorganisms [10].

Therefore the present study is aimed at identifying a suitable solvent in extracting organic bio compounds and need for continuous search for antimicrobial agents, which plants offer a variety of such. The scope of this study is limited to only four (4) different solvents and *V. unguiculata* leaf for the extraction and determination of phytochemicals and antimicrobial activity of the leaf extracts.

Materials and methods

Study Area

The fresh leaf of *V. unguiculata* was collected from Tinto-lawanti Akko, Akko Local Government, Gombe state of Nigeria. The leaf sample was identified by a botanist/taxonomist at Department of Biological Sciences Gombe State University. The leaf obtained and identified was allowed to dry sufficiently under shade at room temperature, after which it was finely powdered using pestle and mortar. The voucher specimen was deposited and kept in good condition for the subsequent analysis.

Preparation of extract

The Finely powdered plant material (500 g) was homogenized with 500 ml of the solvents for extraction using cold maceration for 24 hours and then filtered. This process was repeated three times. The filtrate was concentrated to dryness at reduced pressure with a rotary evaporated. The resultant residues were later dissolved with the respective solvent to 100 mg/ml. In the case of the antibacterial tests, the ethanol extract was re-dissolved using dimethyl sulphoxide. This method was repeated for the ethanol, n-Hexane, ethyl acetate and aqueous extracts of the plant sample. Two-fold serial dilution was carried out to produce the following concentration ranges respectively; 1000, 500, 250 and 125 µg/l.

Preparation of microorganisms

Clinical isolates of *Echerichea coli, Shigella dysentrae, Salmonella typhi ,Klapsela pneumoniae, Helicobacta pylori, Pseudomonas aurigenosae and Staphyloccoci aureous* was obtained from Federal Teaching Hospital Gombe following all ethical requirements. Biochemical test and examination was carried out to confirm and authenticate the bacterial isolates in comparison with available literatures. Using aseptic techniques a single colony was transferred into a 10 ml bottle of nutrient broth, capped and placed in incubator overnight at 35^{o}C. After 12-18 hr of incubation, using aseptic preparation and with the aid of a centrifuge, a clean sample of bacterial strain was prepared. The clinical isolate was sub-cultured in a fresh media so that the bacterial strain will remain active before use.

Preliminary phytochemical screening

Phytochemical examination was carried out for the different leaf extracts of *V. unguiculata* to determine the presence of Alkaloid, Glycoside, Saponin, Flavonoid, Phytosterol, Steroid, Phenol, Anthraquinones, Tannin and Carbohydrate according to the standard methods reported by [11-13].

Antimicrobial assessment

The antimicrobial assessment was tested by using Agar well diffusion method as described

by [14]. The antimicrobial activity of the crude leaf extract *V. unguiculata* was determined using some human pathogens/Clinical bacterial isolates which was obtained from Federal Teaching Hospital Gombe. The microbes obtained was examine to establish its purity by Microbiology Department of Gombe State University, Thereafter, All the pure culture of the test organisms was sub cultured for 24 hours and 2 hours in broth agar respectively. 10 g of the extracts was weighed each and each and was dissolved in 10 ml of Di-methyl sulfoxide (DMSO) to obtain a concentration of 100 mg/ml of the extracts. Mueller Hinton agar was used as the growth medium for the microbes. The medium was prepared according to manufacturer's instructions. The medium was sterilized at 12^{o}C for 15 min and was poured into sterilized petri dishes, the plates was allowed to cool and solidify. Diffusion method was used for screening the extracts. The sterilized and solidified media was seeded with 0.1 ml of the standard inoculums of the test microorganisms. Using standard cork-borer of 6 mm in diameter to make four (4) wells around each inoculated medium. 1000 µg/l, 500 µg/l, 250 µg/l, and 125 µg/l of the extracts of concentration 100 mg/ml was then inoculated into the cut wells on the seeded medium. The inoculated plates was then incubated at 37^{o}C for bacteria and 27^{o}C for 24 hr (standardized) after which the plates was observed for zones of inhibition of growth. The zones was measured and the results recorded in mm. based on the criteria for the interpretation of the in vitro susceptibility of microorganisms when exposed to the antimicrobial agent, comparing it with established standards by the NCCLS. An extract is considered active when the inhibition zone diameter is higher than 6 mm [15].

Result and discussion

Results reported in Table 1 shows the presence of most of the bioactive compounds in ethanol, ethyl ether, n-hexane and aqueous extracts of *Vigna unguiculata.* These bioactive compiunds are found as Saponins, tannins, alkaloids, steroids, glycosides, phenolic compound, anthraquinone, photosterols and carbohydrate were all present in the extracts of the leaf of *Vigna unguiculata*while flavonoids is absent in ethanol extract, flavonoids and anthraquinones were also absent in aqueous solvent. The result shows that the four (4) different solvents have potential for crude extraction in cold maceration. The slight difference in the results obtained with other researchers could be due to the part of the plant used, age of the plant, percentage humidity, climatic conditions, soil conditions, geographical locations, and time of harvesting or method of extraction [16].

Table 1. Phytochemical screening of ethanolic extract of *Vigna unguiculata*

Bioactive compounds	Ethanol extract	Ethyl-ether extract	n-Hexane extract	Aqueous extract
Saponins	+	+	+	+
Glycosides	+	+	+	+
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	+	+	+	_
Volatile oils	+	+	+	+
Phenolic compound	+	+	+	+
Anthraquinone	+	+	+	_
Steroids	+	+	+	+
Carbohydrate	+	+	+	+

Key: (+) Compound is present, (–) = Compound is absent

The chemical constituents present in the extracts have some biological activity, like phenol, flavonoids, tannins, quinines which exhibit antimicrobial, anti-carcinogenic and antioxidant activities [17].

Antimicrobial activity of the extracts of the different solvents of V. unguiculata

The antibacterial activity of the four different solvents (ethanol, n-Hexane, ethyl acetate and aqueous) shows reasonable zone of inhibition against tested Microorganisms (Table 2-5) using

6 mm diameter size of cup borer. The highest zone of inhibition demonstrated by the solvents was obtained by ethanol extract for *H. pyroli* (19 mm) and *P. aeruginosa* (14 mm) while *E. coli* for n-Hexane extract (14 mm) The ethanol extract showed the highest zone of inhibition (19 mm) on H. pyroli than the other solvents. The results of this work agrees with the work of [18] that the highest zone of inhibition produced by the ethanol extract demonstrate that ethanol was a better extracting medium for the phytochemical with antimicrobial activity. An inhibition zone of 8 mm or greater was considered as a good antimicrobial activity [18]. A cleared zone bigger than 7 mm was interpreted as sensitive while smaller than 6 mm was interpreted as resistance extract using 6 mm cork-borer size of the well diameter [19].

Table 2. Zone of inhibition (mm) for ethanol extract of *V. unguiculata*

Conc. of ethanol extract (µg/l)	*S. dysenteriae*	*E. coli*	*S. typhi*	*K. pneumoniae*	*H. pyroli*	*P. aeruginosa*	*S. aureus*
1000	10	13	11	12	19	14	11
500	9	12	11	12	14	10	11
250	9	11	9	12	12	8	10
125	8	10	9	10	10	8	8
200 Amoxicilin (control)	20	21	16	20	25	15	16

Table 3. Zone of inhibition (mm) for n-Hexane extract of *V. unguiculata*

Conc. of n-hexane extracts (µg/l)	*S. dysenteriae*	*E.coli*	*S. typhi*	*K. pneumoniae*	*H. pyroli*	*P. aeruginosa*	*S. aureus*
1000	12	14	8	11	12	8	11
500	11	12	8	11	11	8	10
250	11	12	8	10	11	8	9
125	10	10	8	10	8	7	8
200 Amoxicilin (control)	20	19	22	14	28	17	17

Table 4. Zone of inhibition (mm) for ethyl acetate extract of *V. unguiculata*

Conc. of aqueous extracts (µg/l)	*S. dysenteriae*	*E. coli*	*S. typhi*	*K. pneumonia*	*H. pyroli*	*P. aeruginosa*	*S. aureus*
1000	11	11	11	11	11	12	12
500	10	10	11	10	10	11	11
250	10	10	11	10	10	10	11
125	9	8	9	10	10	10	10
200 Amoxicilin (control)	22	18	20	15	29	18	16

Table 5. Zone of inhibition (mm) for aqueous extract of *V. unguiculata*

Conc. of ethyl acetate extracts (µg/l)	*S. dysenteriae*	*E. coli*	*S. typhi*	*K. pneumoniae*	*H. pyroli*	*P. aeruginosa*	*S. aureus*
1000	13	12	11	11	11	11	10
500	10	11	10	11	11	10	10
250	9	11	10	10	10	10	9
125	9	10	10	9	10	9	9
200 Amoxicilin (control)	20	16	20	14	28	17	16

Conclusions

From the results obtained, it is found that ethanol has greater affinity to extract organic bio compounds followed by Ethyl acetate then Aqueous and n-hexane respectively. The antimicrobial activity and the presence of active phytochemical compounds shows the potency of *V. unguiculata* leaf in curing of ailments which supports its use in traditional medicine in Nigeria. Therefore there is need for more active research on this plant leaf as it can be used as an alternative to synthetic drug formulation.

Conflict of interest

Authors of this work declare no conflict of interests.

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