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Phytochemical and Antimicrobial Activities of Extracts from Six Medicinal Plants Utilized as Antimalarials in Ethno-Medicine

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Abstract Six out of forty plants attested to be utilized in antimalarial in South West Nigeria by herbal traders and native doctors were collected. The plants are *Sphenocentrum jollyanum*, *Spathodea campanulata*, *Kigelia africana*, *Harungana madagascariensis*, *Ficus exasperata* and *Antiaris africana*. Their methanol extracts were obtained by cold extraction. Extracts were subjected to phytochemical tests using standard methods. Minimum inhibition concentration (MIC) were determined against five microbes [*Bacillus subtilis*, *Bacillus cereus*, *Proteus mirabilis*, *Salmonella typhi* and *Candida albicans*]; to assess antibacterial and antifungal activities of each extract. All the eighteen extracts contain saponins, almost all have reducing sugars. Five of the plants contain alkaloids and resins. Flavonoids and phenols were moderately present in the extracts. Phlobatannins and anthraquinones were not abundant in the plant extracts. Antibacterial activities of the extracts are more pronounced than their antifungal potentials.

Keywords Secondary metabolites, bacteria, fungi, ethno-medicine, bioactivity, MIC

Introduction

In a recent survey carried out on plants used by herbal traders and native doctors in antimalarial therapies in south-west Nigeria [1], forty plants were identified. Six out of these plants were found to be interesting from literature. The six indigenous medicinal plants are *Sphenocentrum jollyanum* Pierre (Menispermaceae), *Spathodea campanulata* Pal Beauv. (Bignoniaceae), *Kigelia africana* (Lam.) Benth. (Bignoniaceae), *Harungana madagascariensis* Lam. ex Poir (Hypericaceae), *Ficus exasperata* Vahl. (Moraceae) and *Antiaris africana* Engl. (Moraceae). Their indigenous and common names are respectively 'akerejupon, African tulip, African sausage tree, Dragon's blood tree, forest sandpaper fig and false iroko [2-6]. They play prominent roles in ethno-medicine for general medicinal purposes; they are also utilized as spices, food, and industrial raw materials. Their ethno-medicinal uses include anti-diabetic, hepato-protective, antioxidant, molluscicidal, antimalarial, antimicrobial, anti-inflammatory, anthelmintic, antiviral, anti-cholinesterase, anti-hyperglycaemic, anti-arthritic/ homeopathic, anticancer/antitumor and hypotensive activities. They have been used as bio-insecticide and acid corrosion inhibitors, in building and furniture making [5-9].

Sphenocentrum jollyanum have been reported to be potentially safe for oral consumption as well as in the control of blood glucose and total cholesterol levels, along with its toxicity tests [10-15]. *Sphenocentrum jollyanum*, *Spathodea campanulata*, *Kigelia africana*, and *Ficus exasperata* were reported to possess antioxidant activities [8, 10-20]. Five of the plants: *Sphenocentrum jollyanum*, *Spathodea campanulata*, *Kigelia africana*, *Ficus exasperata* and *Antiaris*

africana were active as antimicrobials [21-28]; while *Sphenocentrum jollyanum*, *Spathodea campanulata*, *Kigelia africana*, and *Ficus exasperata* possessed anti-inflammatory activities [20, 29, 30].

Some compounds have been reported to be isolated from different parts of the plants, along with the evaluation of their bioactivities. Columbin, isocolumbin and fibleucin were three furanoditerpenes isolated from fruits of *Sphenocentrum jollyanum*, these compounds were claimed to be responsible for its anti-inflammatory activity [31].

Other compounds reported from different parts of *S. campanulata*, include spathodic acid, ursolic acid, tomentosolic acid and pectic substances from the stem bark [32]. Leaves contain spathodol, caffeic acid, other phenolic acids and flavonoids, while fruits have polyphenols, tannins, saponins and glucosides. The flowers contain anthocyanins while its floral nectar contains a complex mixture of triterpenoids and steroids [32-39]. Naphthaquinones, iridoids, fatty acids, norviburtinal, sterols, lignans, terpenoid, and flavonoids were some of the constituents found in *Kigelia africana* [8]. The major constituents of root essential oil of *S. jollyanum* were α-eudesmol, α-pinene, isocaryophyllene, 1,8-cineole and β-pinene [15].

Leaf and stem essential oils of *Harungana madagascariensis* Lam. ex Poir, were reported to contain sixty-four compounds, dominated by α-farnesene, α- and β-caryophyllenes [40]. Other important phytochemicals have been isolated and reported from *Harungana madagascariensis*, such include harunganin and its derivatives, anthranoids like harongin, anthrone, harunganol B, kenganthranol A and 1,7-dihydroxyxanthone [41, 42].

Asekun *et al*., 2006, reported leaf and flower essential oils of *Kigelia africana* [43].

Triterpenoids and betaines were isolated from the latex and bark of *Antiaris africana* [44]. GC and GC-MS analyses revealed the presence of eucalyptol, isomenthol, linalool and other interesting C10 and C15 terpenoids in leaf, stem-bark and root essential oils of *Antiaris africana* [45]. Xanthone, triterpenes and gamma lactone were the compounds reported in the stem bark of *Antiaris africana* [46].

The six plants being reported here are widely utilized in etnomedicine, but are yet to be fully assessed scientifically. This study is on the phytochemicals and antimicrobial activities of eighteen extracts from different parts of these six plants. Our data will unravel some information on each plant for better applications. This study has not been earlier reported in literature.

Materials and Methods

Plant Collection and Identification

Plants samples of *S. jollyanum, S. campanulata, K. africana, H. madagascariensis, F. exasperata and A. Africana* were collected from Ijebu and Ago-Iwoye, Ogun state, Nigeria. They were identified and authenticated at the Herbarium, Department of Botany, University of Ibadan where voucher specimens were deposited, with numbers UIH-22643; UIH-22493; UIH – 22455.

Preparation and Extraction

Plant samples [500 g to 1 Kg] were cut into small pieces, air-dried and ground to a fine powder. These were extracted using methanol. Each solvent extract was concentrated by rotary evaporator at 40 ^{o}C to give the respective crude extracts.

Phytochemical analysis of extracts

The eighteen plant extracts were subjected to phytochemical screening to determine the presence of the following secondary metabolites, Phlobatannins, tannins, saponins, reducing sugars, alkaloids, resins, terpenoids, steroids, flavonoids, phenols and anthraquinones, using standard methods [47 - 48].

Determination of Antimicrobial Activity

Microorganisms used were standard strains of two gram positive bacteria: *Bacillus subtilis* (ATCC 14579) and *Bacillus cereus* (ATCC 33923); two gram negative: *Proteus mirabilis* (ATCC 21784) and *Salmonella typhi* (ATCC 25179) and a fungus: *Candida albicans* (NCTC 227). These were obtained from Centre for Drug Research Institute (CDRI) Lucknow, India. Standard microbial cultures were prepared by sub-culturing a loopful of each microbe into sterile nutrient broth and incubated for 24 h at 37 ^{o}C for the bacteria and 48 h for the fungus. The suspensions were adjusted to a turbidity of 10^5 colony forming unit (cfu/mL).

Minimum inhibitory concentrations (MICs) were determined using the tube dilution methods. Nutrient broth (2 mL) was dispensed into each of the twelve (12) test tubes followed by an addition of the extract (1 mL) in the first tube. From the resulting mixtures, 0.5 mL of the solution was removed to make serial dilution to the nineth tube excluding the neutral, negative and positive controls. Thereafter, 0.2 mL of each organism was added into the test tubes. Gentamicin (0.3 mL) and Ethylacetate/water, 1:1, were used as the positive and negative controls respectively. The nutrient broth with organisms only was used as the neutral control. Lowest concentration that showed no growth was the MIC after 24 h incubation at 37 ^{o}C.

Results and Discussion

Phytochemical screening

Methanol extracts of the leaf, stem and bark of *S. jollyanum* showed presence of saponins, terpenoids, alkaloids, reducing sugars and cardiac glycoside. Resins occurred only in the leaf and bark extracts while flavonoids were found in the stem and bark extracts. Phlobatannin is not in *S. jollyanum* extracts.

The three extracts of *S. capanulata* have saponins, flavonoids and phenols. Extracts of leaves and roots have steroids and terpenoids and those of leaves and stems possessed cardiac glycoside in addition to the other secondary metabolites. Alkaloids, anthraquinones and phlobatannins were not in the extracts of *S. capanulata*.

Leaf, bark and fruit methanol extracts of *K. africana* gave positive results during screening for tannins, saponins, alkaloids, flavonoids, reducing sugars, phenols and cardiac glycosides. Terpenoids were observed only in fruit and bark extracts and phlobatannins in leaf and bark extracts (Table 1).

Tannins, saponins, alkaloids, reducing sugar, resin and phenol were in the three methanol extracts of *H. madagascariensis* while anthraquinones and steroids were not detected. Terpenoids were observed in leaf and stem extracts, cardiac glycosides in stem extract and phlobatannins in bark extract. *F. exasperata* extracts contain saponins, terpenoids, alkaloids, flavonoids, reducing sugars and resins. They do not have phlobatannins, steroids and anthraquinones. Leaf and root extracts; leaf and bark extracts; bark and root extracts possess tannins, phenols and cardiac glycosides respectively.

Table 1: Phytochemical Screening of Methanol Extracts of the Six Plants

S/N	Ext	Tan	Phl	Sap	Ste	Ter	Alk	Fla		Red	Res	Car		Phe	Ant
								Mtd1	Mtd2			Mtd1	Mtd2		
1	ScL	√	X	√	√	√	X	X	√	X	√	√	√	√	X
2	ScS	X	X	√	X	X	X	X	√	√	√	X	X	√	X
3	ScR	X	X	√	√	√	X	X	√	√	√	X	X	√	X
4	FeL	√	X	√	X	√	√	X	√	√	√	X	X	√	X
5	FeB	X	X	√	X	√	√	X	√	√	√	√	X	√	X
6	FeR	√	X	√	X	√	√	X	√	√	√	√	X	X	X
7	HmL	√	X	√	X	X	√	X	X	√	√	X	X	√	X
8	HmB	√	√	√	X	√	√	X	X	√	√	X	X	√	X
9	HmS	√	X	√	X	√	√	√	√	√	√	√	X	√	X
10	KaL	√	X	√	√	X	√	X	√	√	√	X	√	√	X
11	KaB	√	√	√	√	√	√	X	√	√	X	√	X	√	X
12	KaF	√	X	√	X	√	√	X	√	√	√	√	X	√	X
13	AaL	X	X	√	√	√	√	√	X	√	X	√	√	X	X
14	AaB	√	X	√	√	X	√	√	X	√	X	√	√	√	√
15	AaR	√	X	√	X	√	√	√	√	√	X	√	X	√	√
16	SjL	X	X	√	√	√	√	X	X	√	√	√	√	X	X
17	SjB	√	X	√	X	√	√	√	√	√	√	√	X	√	X
18	SjS	X	X	√	X	√	√	X	√	√	X	√	√	X	√

Keys: √ - Present; Absent – X; ScL (S) (R) - *Spathodea campanulata* Leaves (Stems) (Roots); FeL (B) (R) *Ficus exasperata* Leaves (Barks) (Roots); HmL (B) (S)- *Harungana madagascariensis* Leaves (Barks) (Stems); KaL (B) (S) - *Kigelia Africana* Leaves (Barks) (Fruits); AaL (B) (R)- *Antiaris africana* Leaves (Barks) (Roots) SjL (B) (S) - *Sphenocentrum jollyanum* Leaves (Barks) (Stems); Ext - Extracts; Tan - Tannins; Phl – Phlobatannins; Sap – Saponins; Ste-Steroids Ter - Terpenoids Alk – Alkaloids; Fla – Flavonoids; Red - Reducing sugars; Res – Resins; Car – Cardiac glycoside; Phe – Phenol; Ant - Anthraquinones

A. africana extracts of leaf, bark and root have saponins, alkaloids, flavonoids and cardiac glycoside. Although, bark and root extracts revealed more groups of metabolites like tannins, anthraquinones and phenols; extracts of leaves and roots indicated presence of terpenoids; and steroids were observed in leaf and bark extracts only (Table 1).

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial drug that will inhibit the visible growth of microorganism after overnight incubation [49]. A lower MIC value indicates that less drug is required for inhibiting growth of the organism; therefore, drugs with lower MIC scores are more effective antimicrobial agents.

Out of the eighteen extracts, leaf, bark and stem extracts of *H. madagascariensis* were strongly active by exhibiting the least MICs at 16 µg/mL, 400 µg/mL and 400 µg/mL respectively against *B. subtilis*. The bark and stem extracts were also the most active against *P. mirabilis*, displaying the lowest MIC at 80 µg/mL compared with other extracts. The three extracts of *A. africana*, two extracts of *K. africana* and two extracts of *S. jollyanum* were most active against *B. cereus*, and showed MIC at 2,000 µg/mL. Leaf and root extracts of *S. campanulata* were the most active against *S. typhi* by exhibiting the lowest MIC at 400 µg/mL. More so, its leaf extract was the only extract, out of the eighteen, that stopped the growth of *C. albicans* at MIC of 400 µg/mL (Tables 2a & 2b)

Table 2a: Minimum Inhibitory Concentration of Extracts of *S. campanulata*, *F. exasperata* and *H. madagascariensis*

Extracts	**Organisms**	**Concentration of extracts in µg/mL**								
		10000	**2000**	**400**	**80**	**16**	**3.2**	**0.64**	**0.128**	**0.0256**
ScL	*B. cereus*	-	+	+	+	+	+	+	+	+
	B. subtilis	-	-	+	+	+	+	+	+	+
	P. mirabilis	-	+	+	+	+	+	+	+	+
	S. tyhii	-	-	-	+	+	+	+	+	+
	C. albicans	-	-	-	+	+	+	+	+	+
ScS	*B. cereus*	+	+	+	+	+	+	+	+	+
	B. subtilis	-	-	+	+	+	+	+	+	+
	P. mirabilis	-	-	+	+	+	+	+	+	+
	S. tyhii	-	-	+	+	+	+	+	+	+
	C. albicans	-	-	+	+	+	+	+	+	+
ScR	*B. cereus*	-	-	+	+	+	+	+	+	+
	B. subtilis	-	-	+	+	+	+	+	+	+
	P. mirabilis	-	-	-	+	+	+	+	+	+
	S. tyhii	-	-	-	+	+	+	+	+	+
	C. albicans	-	-	+	+	+	+	+	+	+
FeL	*B. cereus*	-	+	+	+	+	+	+	+	+
	B. subtilis	-	+	+	+	+	+	+	+	+
	P. mirabilis	+	+	+	+	+	+	+	+	+
	S. tyhii	-	-	+	+	+	+	+	+	+
	C. albicans	-	-	+	+	+	+	+	+	+

FeB	*B. cereus*	-	-	+	+	+	+	+	+	+
	B. subtilis	-	+	+	+	+	+	+	+	+
	P. mirabilis	+	+	+	+	+	+	+	+	+
	S. tyhii	-	-	+	+	+	+	+	+	+
	C. albicans	-	-	+	+	+	+	+	+	+
FeR	*B. cereus*	-	+	+	+	+	+	+	+	+
	B. subtilis	-	+	+	+	+	+	+	+	+
	P. mirabilis	+	+	+	+	+	+	+	+	+
	S. tyhii	-	+	+	+	+	+	+	+	+
	C. albicans	+	+	+	+	+	+	+	+	+
HmL	*B. cereus*	-	+	+	+	+	+	+	+	+
	B. subtilis	-	-	-	-	-	+	+	+	+
	P. mirabilis	-	-	+	+	+	+	+	+	+
	S. tyhii	-	-	+	+	+	+	+	+	+
	C. albicans	+	+	+	+	+	+	+	+	+
HmB	*B. cereus*	-	-	+	+	+	+	+	+	+
	B. subtilis	-	-	-	+	+	+	+	+	+
	P. mirabilis	-	-	-	-	+	+	+	+	+
	S. tyhii	-	-	-	+	+	+	+	+	+
	C. albicans	-	-	+	+	+	+	+	+	+
HmS	*B. cereus*	-	+	+	+	+	+	+	+	+
	B. subtilis	-	-	-	+	+	+	+	+	+
	P. mirabilis	-	-	-	-	+	+	+	+	+
	S. tyhii	-	-	+	+	+	+	+	+	+
	C. albicans	+	+	+	+	+	+	+	+	+

Key: + = Growth; - = No growth

S. campanulata L-leaf, S-stem, R-root; F. exasperata L-leaf, B-bark, R-root; and *H. madagascariensis L-leaf, B-bark, S-stem*

Table 2b: Minimum Inhibitory Concentration analysis of Extracts of *Kigelia africana*, *Antiaris africana* and *Sphenocentrum jollyanum*

Extracts	**Organisms**	**Concentration of extracts in µg/mL**								
		10000	**2000**	**400**	**80**	**16**	**3.2**	**0.64**	**0.128**	**0.0256**
KaL	*B. cereus*	+	+	+	+	+	+	+	+	+
	B. subtilis	-	-	+	+	+	+	+	+	+
	P. mirabilis	+	+	+	+	+	+	+	+	+
	S. tyhii	-	-	+	+	+	+	+	+	+
	C. albicans	+	+	+	+	+	+	+	+	+
KaB	*B. cereus*	-	-	+	+	+	+	+	+	+
	B. subtilis	-	-	+	+	+	+	+	+	+
	P. mirabilis	-	+	+	+	+	+	+	+	+
	S. tyhii	-	-	+	+	+	+	+	+	+
	C. albicans	-	-	+	+	+	+	+	+	+
KaF	*B. cereus*	-	-	+	+	+	+	+	+	+
	B. subtilis	-	-	+	+	+	+	+	+	+
	P. mirabilis	-	-	+	+	+	+	+	+	+
	S. tyhii	-	-	+	+	+	+	+	+	+
	C. albicans	-	-	+	+	+	+	+	+	+
AaL	*B. cereus*	-	-	+	+	+	+	+	+	+

	B. subtilis	+	+	+	+	+	+	+	+	+
	P. mirabilis	+	+	+	+	+	+	+	+	+
	S. tyhii	+	+	+	+	+	+	+	+	+
	C. albicans	-	-	+	+	+	+	+	+	+
AaB	*B. cereus*	-	-	+	+	+	+	+	+	+
	B. subtilis	+	+	+	+	+	+	+	+	+
	P. mirabilis	-	+	+	+	+	+	+	+	+
	S. tyhii	+	+	+	+	+	+	+	+	+
	C. albicans	**?**	+	+	+	+	+	+	+	+
AaR	*B. cereus*	-	-	+	+	+	+	+	+	+
	B. subtilis	-	-	+	+	+	+	+	+	+
	P. mirabilis	-	-	+	+	+	+	+	+	+
	S. tyhii	-	-	+	+	+	+	+	+	+
	C. albicans	+	+	+	+	+	+	+	+	+
SjL	*B. cereus*	+	+	+	+	+	+	+	+	+
	B. subtilis	-	-	+	+	+	+	+	+	+
	P. mirabilis	-	-	+	+	+	+	+	+	+
	S. tyhii	-	-	+	+	+	+	+	+	+
	C. albicans	+	+	+	+	+	+	+	+	+
SjB	*B. cereus*	-	-	+	+	+	+	+	+	+
	B. subtilis	-	-	+	+	+	+	+	+	+
	P. mirabilis	-	-	+	+	+	+	+	+	+
	S. tyhii	-	-	+	+	+	+	+	+	+
	C. albicans	-	-	+	+	+	+	+	+	+
SjS	*B. cereus*	-	-	+	+	+	+	+	+	+
	B. subtilis	-	-	+	+	+	+	+	+	+
	P. mirabilis	-	-	+	+	+	+	+	+	+
	S. tyhii	-	-	+	+	+	+	+	+	+
	C. albicans	-	-	+	+	+	+	+	+	+

Key: + = Growth; - = No growth

K. africana L-leaf, B-bark, F-fruit; A. africana L-leaf, B-bark, R-root and S. jollyanum L-leaf, B-bark, S-stem

All of the eighteen extracts were found to have saponins. Pure saponin extract have been reported to exhibit remarkable antimicrobial activity against *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger* [50]. Alkaloids, present in the six plants except *S. campanulata*, were reported to have displayed antimicrobial properties [51-52]. Four out of the six plants revealed flavonoids in all their parts. Many flavonoids have anti-inflammatory, free radical scavenging, hepatoprotective and anticancer activities [53-54]. Many terpenoids are biologically active and have been used in the fight against cancer, malaria, inflammation, and a variety of infectious diseases [55]. Other secondary metabolites such as tannins [56], phenols [57], resins [58], reducing sugar, steroids and cardiac glycosides would have acted in synergy with the aforementioned groups to achieve various observed bioactivities of different plants parts, which is utilized in ethno-medicine.

Conclusion

The eighteen methanol extracts from the six plants were evaluated for their phytochemical and antimicrobial properties using standard methods. They all contain saponins and reducing sugars except *Spathodea campanulata* leaf. Five of the plants contain alkaloids and resins. Terpenoids were more abundant than steroids in these plants, while flavonoids and phenols were moderately present among the extracts. Phlobatannins and anthraquinones were

not common in the plant extracts. Phlobatannins are in *H. madagascariensis* and *K. africana* while anthraquinones were present in only *A. africana* and *S. jollyanum*.

Antibacterial activities of extracts were more pronounced than their antifungal potentials. Fungal growth inhibitory activity of *Spathodea campanulata* leaf methanol extract is dose dependent with MIC value of 400 µg/mL when compared to the standard (gentamicin). The six indigenous medicinal plants are utilized widely in ethno-medicine. Our report on their phytochemicals and antimicrobial assessments justify their ethno-medicinal applications. This study has not been reported in literature.

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