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Chemical, Phytochemical and Antimicrobial Screening of Extracts of *B. sapida* for Agricultural and Medicinal Relevance

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Abstract: Phytochemical screening of the fruit of *Blighia sapida* confirmed the presence of Saponin, Saponin glycoside, Tannin, Balsam, Cardiac glycoside and Volatile oil. Spectrophotometric analysis for trace metals (such as Mn, Zn, Cu, Ni and Fe), Phosphorus and Sulphur showed that *B. sapida* contained Mn (0.332±0.003 mg/100g), Zn (1.820±0.001 mg/100g), Cu (0.253±0.002 mg/100g), Ni (1.074±0.001 mg/100g), Fe (0.791±0.002 mg/100g), Pb (0.010±0.001), P (49.20±0.200 mg/100g) and S (719.83±0.290 mg/100g). The medicinal and agricultural relevance of the extracts were evaluated in-vitro by antimicrobial and antifungal assays. The aqueous extract (but not methanol and petroleum ether extracts) showed growth inhibitory effects on *Staphylococcus aureus* and *Escherichia coli*, but *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* were resistant to all the plant extracts and the antibiotic controls. The Minimum Inhibitory Concentration (MIC) of the aqueous extract of *B. sapida* on *S. aureus* and *E. coli* were 3.13 mg and 12.50mg respectively. The Minimum Bacterial Concentration (MBC) of the aqueous extract against the test organisms ranged from 12.50mg to 25.00mg.
[John-Dewole OO, Popoola OO. **Chemical, Phytochemical and Antimicrobial Screening of Extracts of *B. sapida* for Agricultural and Medicinal Relevance.** *J Am Sci* 2019;15(8):42-47]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org. 7. doi:10.7537/marsjas150819.07.

Keywords: antimicrobial; *B. sapida;* herbal; pharmaceutical; phytochemical

1. Introduction

The use of plants and plant extracts for medicinal purposes has been going on for thousands of years; it has also form the source of much useful therapy in both herbalism and folk medicine (Sofowora, 1999). The use of medicinal plants in traditional medicine has also generated a lot of interest and concern about their efficacy and safety margin, since 65-70% of the Nigerian population patronizes traditional medicine practitioners in their various forms and methods (Bubayero, 1998 and Sofowora, 2001). Plants produce many chemical compounds that are having various potential values in the treatment of diseases, but a number of them could also be poisonous. Chemical compounds with beneficial effects have been isolated and biologically assayed to establish their medicinal activity. Modern drugs used in orthodox medicine have also been sourced from plants (Sofowora, 2001). It is therefore not surprising that medicinal plants are vastly employed in the treatment of various ailments which include; snake-bite, eye injuries, conjunctivitis, burns, scalds, abdominal colic, peptic ulcer, diarrhea, dysentery, chronic ulcer, measles, hepatitis, arthritis and rheumatism (Esuoso and Odetokun, 2005). Mere isolation and elucidation of chemical structures of plant extracts may not be too significant, until appropriate bioassays are carried out to establish the biological activity exhibited by the plant extract (Ekong, 2006). *B. sapida*, also known as 'Akee apple', belongs to the plant family called *Sapindaceae* and it is noted for its highly distinctive reddish fruits. There are different species of this plant, which include *Blighia sapida*, *Blighia welwitschii* and *Blighia unijugata* (Keay, 1999). *B. sapida* is a familiar tree often planted to provide shade from hot sun. It is known locally as 'isin' in Yoruba, 'gwanja kusa' in Hausa and 'okpu' in Igbo (Keay, 1999). *B. sapida* is about 25m high and 2.5m in girth, with a heavy evergreen crown. The bark is pale brown, while the leave has a stout stalk of about 5–23cm long. The leaflets, 5-15cm long by 3.5-7.5cm broad, are obovate with the lowest part almost circular and close to the base of the leaf-stalk (Keay, 1999). Flowering of the plant begins between October – March. The flowers are small and greenish white in colour. The fruits start appearing between March to September. The fruits are obovoid and about 3.5-6cm long by 3-5cm in diameter, bright red to yellowish in colour and often split open on the tree. The seed is covered with a glossy testa and about 2.5cm long by 2cm broad, while the aril (i.e. the edible part of the fruit) is pale yellow or cream coloured, wrinkled and about 2cm long (Keay, 1999). *B. sapida* is a native of West Africa. It extends from Senegal to Gabon. It is also cultivated in India and tropical America.

B. sapida is well distributed throughout Nigeria and found in drier forest of the savannah region (Esuoso and Odetokun, 2005). *B. sapida* is a medicinal plant commonly used by traditional healers in Nigeria, and highly valued in Africa (Owonubi, 2006) for the treatment of various ailments. Okogun (1996) stated that the bark pulp is used as liniment for oedema and intercostal pains in Cote d'Ivoire, while the bark is powdered and grounded with capsicum and rubbed on the body as a stimulant. The ashes of the dried husks and seeds are used in the preparation of soap, because they are rich in potash. The extracts of the leaves are used as eye drop in ophthalmia and conjunctivitis. Locally, various parts of *B. sapida* plants are used either alone or in combination for the treatment of psychosis, cancer, gonorrhea, stomach ache, hernia, backache, diarrhoea and constipation (Okogun, 1996 and Owonubi, 2006). Thus, the aim of this study is to investigate the various chemical, phytochemical and anti-microbial components of the husks of *B. sapida* that are available for medicinal, pharmaceutical and agricultural use.

2. Materials and Methods

The fruits of *B. sapida* were harvested from the tree species found in the College of Forestry, Ibadan in Oyo State. The plant species was later identified and authenticated by the Department of Botany, University of Ilorin, Kwara State. The aril and the seeds in the fruits were removed with a sharp knife and the husks were dried at $32^{o} \pm 2^{o}C$ for two weeks on a clean pavement prior the analysis. The drying process was further enhanced by the harmattan wind.

Sampling

The dried bulk samples of the husks were pulverized using pestle and mortal, and sieved through a $2mm^2$ wire mesh to obtain a fine powder. The powdered samples were mixed together and quartered to obtain a representative sample weighing 150g.

Aqueous Extract

20g of powdered husks of *B. sapida* was weighed into 250ml beaker and 150ml of distilled water was poured unto the beaker content. The solution was stirred with a glass rod and allowed to soak for 24 h. The aqueous extract was filtered thrice through a plug of absorbent cotton-wool in a glass funnel. The aqueous extract was then filtered through 11cm Rundfilter paper MN713. The solution was concentrated by gentle evaporation on a heating mantle and poured into a 100ml beaker.

Methanolic Extract

200ml of methanol was measured into the round-bottom flask of the soxhlet. 20g of the powdered husks of *B. sapida* was placed in the thimble of the soxhlet extractor. The apparatus was coupled and the system was switched on at thermostat temperature of $65^{o}C$. The sample was continuously extracted under reflux for 3 h, and the extract was poured into 100ml flask. Methanolic extract of the sample was concentrated by gentle evaporation on a heating mantle.

Petroleum Ether Extract

200ml of petroleum ether was measured into the round-bottom flask of the soxhlet. 20g of the powdered husks of *B. sapida* was placed in the thimble of the soxhlet. The apparatus was coupled and the system was switched on at thermostat temperature of $60^{o}C$. The sample was continuously extracted under reflux for 3 h and the extract was poured into 100ml beaker after some of the petroleum ether had been recovered. The 100ml extract of the sample was concentrated by gentle evaporation on a heating mantle.

Phytochemical Screening of Crude Extracts

Phytochemical screening of the crude extracts for saponin, saponin glycoside, tannin, anthracene, alkaloid, volatile oil, balsam and cardiac glycoside were carried out by the methods described by Evans (2002) and Sofowora (2001).

Spectroscopic Analysis of Crude Extracts

Methods of Howtz (1999), Skoog et al., (2006) and Pavial et al., (2007) were used for spectroscopic analysis of the samples, using Atomic Absorption Spectrophotometer (A200).

Colorimetric determination of Phosphorus was done using Vanadomolybdate (Yellow) method (AOAC, 2000). Spectrophotometric determination of Sulphate was done using Turbidometric method (AOAC, 2000). Antimicrobial assay of crude extracts of *B. sapida* was done using the methods described by Egwari (1999), Ntiejumokwu and Kolawole (1999), and WHO (1999) to test the effects of crude extracts on the following pathogenic microorganisms: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Saccharomyces cerevisiae*. Determination of antibiotic activity and antibiotic control was done by using the Disc Diffusion and Agar Diffusion techniques as described by WHO (1999). Determination of Minimum Inhibitory Concentration (MIC) of the crude extracts was done by using Tube Dilution method as described by Rotimi et al., (1999).

3. Results

Table 1 gives the phytochemical compounds present in crude extract of the husks of *B. sapida*. The extracts were positive for some of the following compounds; alkaloids, anthracene, balsam, cardiac glycoside, saponin, saponin glycoside, tannin and volatile oil indicating their presence in the extract.

Table 1. Phytochemical Compounds in the husks of *B. sapida*

Phytochemical Compounds	Remarks
Alkaloids	+ve
Anthracene	-ve
Balsam	+ve
Cardiac glycoside	-ve
Saponin	++ve
Saponin glycoside	+ve
Tannin	++ve
Volatile oil	-ve

Key: ++ve = strongly positive, +ve = positive, -ve = negative

Table 2 shows the trace metal contents of the plant extract in mg/100g. The extracts contained Manganese, Zinc, Copper, Nickel, Lead and Iron, while Table 3 shows the concentration of Phosphorus and Sulphur content of the extract in mg/100g.

Table 2. Trace Metals content in mg/100g

Elements	Conc. (mg/100g)
Manganese	0.332±0.003
Zinc	1.820±0.001
Copper	0.253±0.002
Cobalt	ND
Cadmium	ND
Nickel	1.074±0.001
Iron	0.791±0.002
Lead	0.010±0.001

The value represents mean ± SD (N=3), ND = Not Detectable

Table 3. Phosphorus and Sulphur concentration of the extract

Elements	Conc. (mg/100g)
Phosphorous	49.20±0.200
Sulphur	719.83±0.290

The value represents mean + SD (N=3)

Table 4 gives the inhibitory effects of extract of *B. sapida* husks at 15mg. *Staphylococcus aureus* and *Escherichia coli* were sensitive to aqueous extract of *B. sapida* with zone diameters of inhibitions of 14mm and 20mm respectively. *S. aureus, P. aeruginosa* and *E. coli* were all resistant (i.e. shows no growth inhibition) to both methanolic and petroleum ether extracts of *B. sapida*. The active ingredients in the plant extracts seemed more soluble in aqueous medium. The plant extracts exhibited no antifungal effects on *Saccharomyces cerevisiae*. *S. aureus* and *E. coli* were sensitive to the antibacterial effects of Ampicillin trihydrate (15mg) and Tetracycline hydrochloride (15mg) which were used as positive controls, with zone diameter of inhibition of 22mm and 26mm respectively (for ampicillin) and 26mm and 27mm respectively (for tetracycline). However, *P. aeruginosa* showed no growth inhibition on the antibiotic controls. Table 5 and 6 show the Minimum Inhibitory Concentrations (MIC) of the extracts on pathogens; the MIC of extracts of *B. sapida* on *S. aureus* and *E. coli* were 3.13mg and 12.50mg respectively.

Table 4: Inhibitory Effects of Extracts of Leaves of *B. sapida* (15mg) Zone diameter (mm) of growth inhibition

Pathogens	Aqueous	Methanol	Pet. Ether	Ampicillin Control	Tetracycline Control
Staphylococcus aureus	14	0	0	22	26
Pseudomonas aeruginosa	0	0	0	0	0
Escherichia coli	20	0	0	26	27
Saccharomyces cerevisiae	0	0	0	0	0

Table 5: The MIC of *B. sapida* on *S. aureus*

Extract	Concentration (mg/100g)	Growth Indication	MIC (mg/100g)
A1	25.00	Nil	
A2	12.50	Nil	
A3	6.25	Nil	
A4	3.13	+	3.13
A5	1.56	+	

Key: A = Aqueous extract
+ = Positive growth
Nil = No growth

Table 6: The MIC of *B. sapida* on *E. coli*

Extract	Concentration (mg/100g)	Growth Indication	MIC (mg/100g
A1	25.00	Nil	
A2	12.50	Nil	
A3	6.25	+	12.50
A4	3.13	+	
A5	1.56	+	

Key: A = Aqueous extract
+ = Positive growth
Nil = No growth

4. Discussions

The crude extracts of the husks of *B. sapida* was chemically and microbiologically assayed for the presence of phytochemical compounds which could be responsible for their medicinal use in traditional medicine, as anti-amoebic, anti-diarrhea, anti-helminthic and treatment of Broncho-pneumonia (Tona 2008; Sofowora, 1999 and Owonubi, 2006). The study showed (Table 1) that the leaves of *T. diversifolia* contained Saponin, Alkaloids, Saponin glycoside, Tannin and Balsam. This result agrees with similar research done by Kela et al. (1999); Menut *et al.* (2002) and Okogun (1996). These phytochemical compounds have pharmacological effects and have been the basis of chemical synthesis of drugs used in modern medicine responsible for their medicinal use in traditional medicine (Sofowora, 2001) and (Okogun, 1996). Saponins are found in most plants as nitrogen-free glycosides, each consisting of a sapogenin and a sugar molecule. Glycosides are large and varied groups of naturally occurring plant products, characterized, on hydrolysis, by the formation of sugar and non-sugar moiety. Schuster et al. (1999) and Egwari (1999) have isolated steroidal glycosides such as Hecogenin, Progesterone, Testerone and Diosgenin from plants and are now being used therapeutically as hormones and contraceptives in medicine. Evans (2002) reported that the fruit *B. sapida* contains saponins which are haemolytic and probably toxic, but Baruah et al., (2000) reported that the toxic agent is neither saponin nor alkaloids. They concluded that the toxic compound is a water soluble substance that is stable at 100^{o}C and is not precipitated by ethanol. The toxic compounds were named hypoglycine A and B. Ekong (2006) also reported that the ingestion of unripe fruit walls, seeds and white aril of *B. sapida* causes `vomiting sickness' that is characterized by marked hypoglycemia and a mortality rate ranging from 40-80%. Bello (1999) reports that there is a high content of hypoglycine A in immature fruit of *B. sapida* while Tongma *et al*., (2008) used Reversed Phase Liquid Chromatography for the determination of hypoglycine A in a canned ackee fruit samples. The high mortality rate associated with the ingestion of unripe ackee fruits was further confirmed by Kela et al., (1999). They reported the high epidemic of fatal encephalopathy in preschool children in Burkina Faso associated with the consumption of unripe ackee fruit. The poisoned children were observed to have common symptoms of hypothermia, vomiting, convulsion and coma. Apart from those symptoms observed in school children, Bello (1999) reported that extract of *B. sapida* produces leucopenia and thrombocytopenia in mice. The toxic property in *B. sapida* has been advantageous in formulations of various insecticides and pesticides. Kela *et al.*, (1999) reported the molluscicidal activity of *B. sapida*, while Ntiejumokwu and Kolawole (1999) reported the pesticidal activity of *B. sapida* and other selected plants. Cardiac glycosides, digitoxin and digoxigenin have varying effects in the cardiovascular systems of human. They are used in the treatment of heart disorders and high blood pressure (Groth, 1994 and Stenlake, 1997). Tannins are polyphenolic compounds also used for medicinal purposes e.g. catechol,

hydroquinine and resorcinol are phenolic salicylates used as analgesics, antipyretics and as internal antiseptics in medicine and surgery (Bello, 1999 and Stenlake, 1997). This research however showed that *B. sapida* has growth inhibitory effect on *S. aureus* and *E. coli*. The toxic property in Hypoglycine A isolated from *B. sapida* may be responsible for the antibacterial activity of this plant. Thus, the presence of these phytochemical compounds in *B. sapida* could be responsible for the observed pharmacological effects and their medicinal use in traditional medicine.

The seed and seed-oil of matured *B. sapida* have some beneficial uses. Esuoso and Odetokun (2005) reported that the seeds and seed-oils of *B. sapida* are rich sources of protein, carbohydrate, fatty acid and amino acid, which could be used in animal feed formulations (Akobundu and Agyakwa, 1997).

Trace Elements are essential components of the body enzymes, haemoglobon, vitamin B_{12} and thyroxin which are important for life processes and metabolism; and are sourced mainly from plants. Analysis of trace metals, sulphur and phosphorus content revealed Mn (0.332±0.003 mg/100g), Zn (1.820±0.001 mg/100g), Cu (0.253±0.002 mg/100g), Ni (1.074±0.001 mg/100g), Fe (0.791±0.002 mg/100g), Pb (0.010 ± 0.001), P (49.20±0.200 mg/100g) and S (719.83±0.29 mg/100g) (See Table 2 & 3). The concentration (mean±SD) of the elements analyzed showed Sulphur > Phosphorus > Zinc > Nickel > Iron > Manganese > Copper > Lead. The high concentration of sulphur could be responsible for the plant's antimicrobial properties. The clinical effectiveness of sulphanilamides in the control of bacterial infection has led to their effective use against pneumonia and *streptococci* infection (Groth, 1994 and Stenlake, 1997). Sulphanilamides interfere with the synthesis of folic acid, and important bacterial growth factor, by utilization of *para aminobenzoic acid* (PABA) necessary for the synthesis of trihydrofolic acid. The study showed that aqueous extracts of the husks of *B. sapida* possess antimicrobial effect against the growth of pure isolates of *S. aureus* and *E. coli*. This is similar to what is reported by Egwari (1999). The result of zone diameters of inhibition of the plant extract on the growth of *Staphylococcus aureus* and *Escherichia coli* (Table 4) compared favourably with that of standard antibiotic controls consisting of *Tetracycline hydrochloride* (15mg) and *Ampicillin trihydrate* (15mg) (WHO, 1999 and Cheesebrough, 2000). *P. aeruginosa* was resistant to all the plant extract and antibiotic controls. This observation agrees with that of Timothy and Nelson (1992) and Cheesebrough (2000). The plant extract had no antifungal activity against *Saccharomyces cerevisiae*.

5. Conclusion

The phytochemical screening of the husk extracts of *B. csapida* tested positive for the presence of alkaloids, saponin, saponin glycoside, tannin and balsam. The concentration (mean±SD) of elements analysed in mg/100g showed S > P > Zn > Ni > Fe > Mn > Cu > Pb. The high concentration of sulphur and phosphorus is an index for the plant's medicinal properties. The medicinal properties of the plant as evaluated *in-vitro* by antimicrobial assay revealed that aqueous extract showed growth inhibitory effects on *S. aureus* and *E. coli*. However, *Pseudomonas aeruginosa* was resistant to the plant extract and antibiotic controls. The plant extracts have no antifungal effects on *Saccharomyces cerevisiae.*

Recommendations

Further work is recommended on isolation and characterization of active chemical compounds responsible for the antimicrobial/antibacterial properties of the plant. The antibacterial effect of the methanol extract and the antifungal effects of the plant extract should be re-evaluated. Medicinal plants are also known to exhibit seasonal variation in chemical properties and bioactivity, which could also affect their medicinal properties at any given period of time. Therefore, there should be an investigation to mitigate the seasonal chemical properties variation of this plant.

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8/16/2019