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Full Length Research Paper

Phytochemical screening and proximate composition of *Phyllanthus amarus*

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ABSTRACT

The nutritional and anti-nutritional benefits of plants provide a better support for human well being. Phytochemical and proximate composition of *Phyllanthus amarus* was investigated in this studies. Both metholic and aqueous extract of *Phyllanthus amarus* revealed the presence of alkaloid, tannins, saponin, flavonoid, reducing sugar, terpenoid, cardiac-Glycoside is however absent in the aqueous extract. The percentage proximate composition; moisture (37.40), crude fat (5.6), crude protein (1.54), ash (9.0), crude fiber (14.99), and carbohydrate (31.47).

Key-words: *Phyllanthus amarus*, traditional uses, Phytochemical constituents, proximate compostion.

INTRODUCTION

Products from natural sources are gradually replacing synthetic drugs all over the world due to that synthetic drugs with time, tends to exhibit adverse effects on the users, unlike drugs produced using medicinal plants which are said to be less toxic and safer than synthetic drugs (Khanna *et al.*, 2002). Plants contain constituents that are bioactive and thus are used in the treatment of many human diseases (Santos *et al.,* 1990).

Phyllanthus amarus is a plant of the family Euphorbiaceae and has about 800 species which are found in tropical and subtropical counties of the world (Mazumba *et al*., 2006 and Tashseen *et al*., 2013). *Phyllantus amarus* has undergone extensive phytochemical research spanning over four decades. Its Spanish name chanca piedra, which means “stone breaker” or “ shatter stone” describes its folkloric use among Amazonians for eliminating gallstones and kidney stones. Besides kidney stones, it is also used for hepatitis, colds, flu, tuberculosis, malaria ,diabetes, hypertension and liver diseases among others. Human and animal studies using a simple tea infusion showed the plant’s ability to promote kidney stone elimination (Santos, 1990). An extract exhibited potent inhibitory effect on calcium oxalate formation in vitro (Campos *etal.,* 1999). *Phyllantus amarus* has been reported to stimulate bile acid secretion and help lower blood cholesterol levels (Khanna *etal.,* 2002). Hydroalcoholic extract of the plant exhibited analgesic effects in mice (Santos *etal*., 1995) and in other newly-tested neurogenic pain models (Santos., 2000). Geraniin contained in these plants is seven times more potent as an analgesic than aspirin or acetaminophen (Miguel *etal.,* 1996); it has demonstrated antiulcerogenic and gasttroprotective effects (Hung *etal.,* 1995). The methanol fraction was found to have hepatoprotective properties (Ahmad *etal.,* 2002). Administration of *phyllantus amarus* extracts to children with acute hepatitis restored liver function within five days (Hung*etal.,* 1995) and indigestion of powdered herbs by adults with chronic hepatitis showed antihepatotoxic effect (Santos*etal.,* 1999). Capsules of the leaf powder significantly caused reduction in systolic blood pressure, increase in urine volume, and in urine and serum sodium excretion as well as a reduction in blood glucose levels (Khanna*etal.,* 1995) in a human trial. Aqueous extract of *P. amarus* increased the lifespan of mice with liver cancer (Rajeshkumar *etal.,* 2000). Aqueous extract of *P. amarus*

exhibited HIV-1 reverse transcriptase inhibition activity (Ogata *etal.,* 1992).

MATERIALS AND METHOD

All the solvent and reagent used in the study were of Analar grade and, unless otherwise stated were sourced from Pax Herbal Clinic and Research Laboratories, Ewu-Esan, Edo State. Nigeria

Collection of plants material

The plant was collected in July 2015 from Pax Herbal Clinic and Research Laboratorie botanical garden, located at ST. Benedict monastery off Benin-Auchi Express-way, Ewu-Esan, Esan cental local government of Edo State, Nigeria and identified by botanist and confirmed by Taxonomist in the Department of Botany, Ambrose Alli University Ekpoma, Edo State. A voucher specimen was deposited at the Herbarium department of Pax Herbal Clinic and Research Laboratories, Ewu, Edo state. The whole plant was rinsed under running tap and air-dried for two weeks, and then pulverized using a mechanical grinder. The pulverized plant was kept in air-tight cellophane bag until used.

Preparation of extracts

30g each of the pulverized plant was macerated successively in 98% Methanol and water for 48hrs each. The mixture were then filtered and filtrates concentrated using a rotary evaporator. The methanol and water concentrate were evaporated to dryness in water bath. The extracts were stored in an airtight sample bottles and kept in a refrigerator until used.

Phytochemical screening assays:

Phytochemical screening was carried out using standard method as described by Trase and Evans (2010) and Sofowora (1993), briefly as stated below:

Test for Alkaloids

The sample filtrate (3ml) was added to 3ml of 1% Hcl and steam for 30 minutes, then left to cool and finally centrifuged at 3000pm for 10 minutes. The supernant (1ml) was separately mixed with 1ml Drangendroff reagent, Mayers reagent and Wanger's reagent (orange precipitate), Mayer's reagent (creamy precipitate) and Wagner's reagent (reddish brown precipitate) which confirmed the presence of alkaloids.

Test for Tannis

The sample filtrate (2ml) was mixed with 2ml of 0.1% $Fecl_3$ solution. The presence of blue-black coloration indicate (Hydrosable Tannis) and brownish green coloration indicate presence condensed Tannin.

Test for Saponin

The sample filtrate (0.5ml) was mixed with 5ml of distilled water and the mixture shaken vigorously. Persistence Frothing indicate positive result.

Test for Flavonoid

The sample filtrate (2ml) was mixed with 2ml dilute ammonia solution followed by the addition of 1ml of concentrated H_2SO_4. Yellow colouration which disappeared upon standing indicated the presence of flavonoid.

Test for Polysaccharide/starch

The sample filtrate (2ml) was mixed with six (6) drops of iodine solution. The presence of blue-black colouration indicate the presence of polysaccharide/starch.

Test for reducing sugar/Glycoside

The sample filtrate (2ml) was mixed with 2ml fehling's solution followed by steaming for 30minutes. The presence of reddish coloration indicated a presence of reducing sugar after boiling.

Test for Phlobatannin

The sample filtrate (2ml) was mixed with 2ml of 1% HCl followed by steaming. The presence of red deposit at the base of test tube indicated the presence of Phlobatannin.

Test for Terpenoid

The sample filtrate (2ml) was mixed with six drops of Brady's reagent. The presence of yellowish orange colouration indicate positive result.

Test for Cardiac-Glycoside (Keller-killiani test)

The sample filtrate (2ml) was mixed with 2ml of glacial acetic acid followed by the addition of 1ml of 0.1% $FeCl_3$

Table 1 . Phytochemical screening for *Phyllanthus amarus* S.

Parameters	Methanol extract	Aqueous extract
Alkaloid	+++	++
Tannin condensed	+	+
Hydrosable	-	-
Saponin	+	+
Flavonoid	++	+
Polysaccride (starch)	-	-
Reducing Sugar	++	+
Phlobatannins	-	-
Terpenoid	++	+
Cardiac-Glycoside	+	-
Steroid	-	-

Key:
+ = mildly presence of compound
++ = moderately present of compound
+++ = Abundantly present of compound
- = Absence of compound

Table 2. PROXIMATE (%) ANALYSIS OF *Phyllanthus amarus*

Plant	moisture	Crude fat	Crude protein	Ash	Crude fibre	Carbohydrate
Phyllanthus	37.40	5.6	1.54	9.0	14.99	31.47

solution and finally 1ml of concentrated H_2SO_4 was added. The presence of green-blue colouration indicated a positive result.

Test for Steroid

The sample filtrate (0.5ml) was mixed with 0.5ml acetic acid anhydride followed by cooling in an ice bath. This was followed by the addition of 0.5ml of chlorofoam and 1ml concentration H_2SO_4 was carefully added with a pipette until the presence of a reddish-brown ring formed at the separation level between the two liquids, which indicate the presence of steroid.

PROXIMATE ANALYSIS

The proximate analysis of the whole plant of *Phyllanthus amarus* is be determined for Crude protein, crude fiber, ash content, moisture content crude fat and carbohydrate using standard procedures (AOAC, 2005).

RESULTS

The results of phytochemical screening and proximate analysis are shown in table 1 and 2.

DISCUSSION

The result of the phytochemical screening indicates the presence of alkaloid, condensed tannin, saponin, flavonoid, reducing sugar and Terpenoid in both methanol and aqueous. The presence of these metabolites suggests great potential for the plant as a source of useful phytomedicines. For instance , the presence of flavonoids and resins might be responsible for its use as anti-inflammatory recipe in Chinese folkloric medicine as some flavonoids has anti-inflammatory effect on both acute and chronic-inflammation (Miguel *etal.,* 1996). Some plants that possess alkaloids are known for decreasing blood pressure and balancing the nervous system. The presence of tannins could also shows that it is an astringent, help in wound healing and anti-parasitic. The presence of terpenes suggests its possible use as anti-tumor and anti-viral agent as some terpenes are known to be cytotoxic to tumor cells. Alkaloids are known to possess anti-malaria property, hence the plant may be a good source of antimalarial for which it is traditionally uses in locally (Santos, 1990). Plant containing saponins are believed to have antioxidants, anti-cancer, anti-inflammatory, and anti-viral properties.

The proximate analysis showed moisture content of 37.4%, this show that the dry plant has high chance of microbial attack and the total ash value of 9.0% suggests that the amount inorganic substance in the plant is not too high. Acid insoluble ash valvue was 1.55% which suggests that the soluble inorganic component is quite small and this may portray the plant as a poor source of dietary inorganic salts. The methanol and water extractive values of 7.7% and 15.1% respectively show that water is better solvent of bulk extraction that methanol.

CONCLUSION

The presence of these constituents accounts for the local medicinal uses. It was concluded that the extract of *Phyllanthus amarus* consists of important constituents of pharmaceutical importance.

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