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PHYTOCHEMICAL ANALYSES OF THREE SUDANESE PLANTS FOR THEIR CONSTITUENTS OF BIOACTIVE COMPOUNDS

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ABSTRACT
Phytochemicals found in higher plants are known to contain several kinds of secondary metabolites with profound biological activities. Such plant derivatives represent untapped sources for unlimited applications in public life and industry. These structurally variable compounds also provide new models for synthetic chemical analogues. In pest control, various extracts were proved significant effects on different pests and diseases; hence some commercial pesticides were formulated from certain botanical compounds. This study focuses on phytochemical analyses of different parts extracts prepared from three indigenous medicinal plants. These were; *Acacia nilotica* (leaves & fruits), *Artemisia annua* (leaves & whole plant) and *Nerium oleander* (leaves & flowers). Three extracts (water, ethanol and petroleum ether) were analyzed through chemical means from each plant part to ascertain their active chemical groups of non polar and polar components. The results of water extracts showed that alkaloids, saponins and flavones are the main chemical groups present in all plants at variable levels. However, ethanol extracts showed all the eight investigated chemical groups distributed in different plant parts and species. In contrast, petroleum ether extracts yielded principally triterpenoids and sterols. Since these plants are available in the country and having many traditional uses, particularly in medicine and pest control, they can be exploited commercially in obtaining such chemicals for different purposes.

KEYWORDS: Phytochemical; secondary metabolites; active compounds; Sudan.

INTRODUCTION

Some herbal products such as nicotine obtained from tobacco *Nicotiana tabacum* leaves, anabasin and lupinine, the alkaloids extracted from Russian weed *Anabasis aphylla*, rotenone from *Derris elliptica* and pyrethrums from *Chrysanthemum cinerariaefolium* flowers have been used as natural insecticides even before the discovery of synthetic organic insecticides (Combell *et al.*, 1993). However, the discovery, development and use of synthetic organic chemicals during the last century have overshadowed the use of herbal products, and since then the pest control approach has been almost completely based on synthetic pesticides (Potter and Beavers, 2005). But, the extensive and indiscriminate uses of pesticides have resulted in serious drawbacks. The evolution of resistant strains to pesticides has become more costly, in addition to the growing concern about the pesticidal toxic hazards to man, livestock and wild life (Bay, 1976). Residues of some persistent chemicals in the environment have subsequently disturbed the ecosystems (Hill, 1989). Sporadic investigations on water resources in Sudan indicated the presence of measurable residual amounts of organochlorine and organophosphorus pesticides in surface water (UNESCO, 2000).

Based on the above mentioned problems of pesticides, researchers all over the world are working hardly to find environmentally safe alternatives. Among the different alternatives highlighted plant extracts have received great attention during last decades as potent sources of safe natural biocides (Whitehead and Bowers, 1983; Ahmed *et al.*, 1984 and Schmutterer, 1990). Several natural products have shown selective actions against a number of pests through a variety of biological activities including production of behavioral modifying chemicals (growth regulators) such as pheromone analogues, repellents, attractants and antifeedants, besides the direct toxicant effects (Bower *et al.*, 1976).

Botanical pesticides are relatively harmless to natural enemies, pollinators and other non-target organisms and present little risk to users and consumers (Bay, 1976; Schmutterer *et al*., 1995 and Satti *et al*., 2004). Therefore, phytochemicals obtained from plants with proven pest control potential can be used as alternative compounds to synthetic insecticides or along with other insecticides under integrated management systems (Schmutterer, 1990). Plant products can be obtained either from the whole plant or by extraction from specific part using different types of solvents depending on the polarity of the intended phytochemicals (Harbone, 1983, and Edriss *et al*., 2008). However, the Sudan with its variable geographical regions is rich with indigenous flora which represent promising reservoir of biocidal compounds that can be used as effective components in integrated pest management, though, no plant species has yet shown a wide scale of practical utility (Satti *et al*., 2010). Accordingly, a programme was put forward, aiming to find suitable botanical materials for controlling different agricultural pests as well as pests of public health. As a part of such programme, this study was proposed to investigate the phytochemical constituents of three prevailing plant species through chemical analytical approach.

MATERIALS AND METHODS

Plant samples and extracts preparation:

Different botanical parts of three plant species were investigated in this study for their constituents of secondary metabolites. These were: *Acacia nilotica* (leaves and fruits), *Artemisia annua* (leaves and whole plant) and *Nerium oleander* (leaves and flowers). The plant materials were collected from Khartoum State, during the rainy season 2009, except those of *A. annua* were brought from the southern region. The freshly collected plant samples were thoroughly washed with clean water and spread on clean papers inside the laboratory to dry under room temperature. Dried samples were made into fine powders using a Laboratory Blender (No. MAH/11/050/0117-Remi Anupam Mixie Ltd.). The powder of each sample was extracted with water and two organic solvents (ethanol and petroleum ether). A soxhlet apparatus was used for organic solvent extraction (Harbone, 1984). Extracts were dried from solvents using a rotary evaporator, and then kept in black bottles and stored in the refrigerator (at 5^oC) until being used for the chemical tests.

Phytochemical analysis

Phytochemical screenings of the prepared extracts for eight secondary metabolites, i.e., alkaloids, saponins, flavonoids, flavones, tannins, amino acids, steroids and triterpenoids, were carried out according to Harbone (1973; 1983; 1988). Hence, the presence or absence of such chemical groups in each extract was tested through chemical analytical means as briefly summarized in the following poits:

Alkaloids: Mayer's reagent was firstly prepared by mixing 7.36g of mercuric chloride and 5.00g potassium iodide in 60ml and 10ml distilled water, respectively, then each completed with water to 100ml volume. A volume of 30 ml aliquots of each prepared extract was evaporated to dryness in an evaporation dish placed in a water bath. 5ml of 2N of HCL was added to the dried extracts and stirred while heating on a water bath for 10 minutes. The extracts were then cooled at room temperature and transferred to test tubes. Few drops of Mayer's reagent were added. Formation of slight or heavy precipitate was taken as presumptive evidence for the presence of alkaloids.

Amino acids: Ninhydrin reagent was prepared by dissolving about 0.2g of ninhydrin in 100ml of ethanol to give 0.2% solution. Two drops of ninhydrin (0.2%) were added to 5ml of each extract; after few minutes a purple-blue colour was appeared indicating the presence of amino acids.

Flavonoids and flavones: Potassium hydroxide solution was prepared by adding 10ml of distilled water to 5.0g of potassium hydroxide in 50ml volumetric flask to give 50% (w/v) potassium hydroxide solution. 20ml aliquot of each extract was evaporated to dryness on a water bath. The residue was dissolved in 30ml of the same solvent used in the preparation of the extract and filtered. The filtrate was used for testing both flavonoid and flavone compounds. In the former case, 1ml of potassium hydroxide solution was added to 3ml of the filtrate in a test tube. A dark yellow colour indicates the presence of flavonoids. Regarding the flavone test, 0.5ml of concentrated hydrochloric acid and few pieces of magnesium turning (as metal) were added to 3ml of the filtrate. The appearance of pink or red colour indicates the presence of flavones.

Tannins: Ferric chloride was prepared by putting 5g of anhydrous ferric chloride in 100ml volumetric flask, where 100ml of distilled water were added to give 5% (w/v) ferric chloride solution. 25ml of the prepared extracts were evaporated to dryness on a water bath. The insoluble residues were stirred with 10ml of hot saline solution, then the mixture was cooled and few drops of ferric chloride test reagent were added to each extract. Formation of a blue-black or a green colour indicates the presence of tannins.

Saponins: The test of saponins was simply done by putting 10ml of aliquots from each extract in a test tube, where 5ml of water were added. The tubes were closed with a cork and shaken vigorously. Formation of foam layer, honey comb in shapes, which remains for a minimum of 30 minutes, indicated the possible presence of saponins.

Sterols and triterpenes: Vanillin reagent is specifically used for testing triterpenoids. It is prepared by dissolving 0.5g of vanillin in 100ml of H_2SO_4 (sulphuric acid). 20ml of the prepared extracts were evaporated to dryness in a water bath and cooled. The residues were collected in 10ml chloroform and dehydrated over anhydrous sodium sulphate. 2.5ml of this solution was mixed with 0.25ml of acetic anhydride acid, followed by one drop of vanillin dissolved in concentrated sulphuric acid. The gradual appearance of green-blue colour was taken as an indication of the sterols, while the pink to purple colour was taken as an evidence for the presence of triterpenes.

RESULTS AND DISCUSSION

Results of presence or absence of the eight tested secondary compounds in all plant samples were presented in three seperate tables for each extract. Clear results were obtained from the various studied parts of the three plants (viz., *Acacia nilotica*, *Artemisia annua* and *Nerium oleander*). The results of water extracts were shown in Table (1). The main compounds in the three plants were alkaloids, saponins and flavones, which found almost in all parts. In addition, tannins, amino acids and triterpenoids were detected in fruits of *A. nilotica*. Flavonoids were absent from all samples.

The results of ethanol extracts were presented in Table (2). The ethanol extracts of *A. nilotica* and *N. oleander* revealed more or less similar results to those obtained by water extracts, but *A. annua* showed different results. Accordingly, the tannins, flavones, alkaloids and amino acids were detected in *A. nilotica*, in addition to flavonoids and steroids, whereas *N. oleander* showed all the chemicals except the flavonoids.

Regarding the results of petroleum ether extracts, triterpenoids and steroids were the chief compounds found in all plants (Table 3). Moreover, *A. nilotica* fruits manifested additional chemicals like tannins, flavones and flavonoids.

The overall picture of the results demonstrated that, saponins, alkaloids, flavones, steroids and triterpenoids were shared by all plants. On the other hand, tannins were dominantly occurred in all extracts of *A. nilotica* fruits. Therefore, the fruits of this tree were known to have many traditional uses in Sudan especially in folk medicine and leather tanning. Pande *et al*. (1981), stated that *Acacia nilotica* seeds contain crude protein, saponin and tannin compounds. Fagg and Greaves (1990) indicated that tannin compounds of *Acacia nilotica* ethanol extract is a powerful molluscicide, algicide and insecticide. Acetone, ethyl acetate and petroleum ether extracts of *Acacia nilotica* showed high larvicidal effects against different mosquito species (Chaubal *et al*., 2005). The petroleum ether extracts of *Acacia nilotica*, *Nerium oleander* and *Artemisia annua* were also reported to exert superior mortality effects as mosquito larvicides (Sharma *et al*., 2006; Tonk *et al*., 2006; Sakthivadivel and Daniel, 2008 and Abdul Rahuman *et al*., 2008).

The plant *Artemisia annua* was found to contain some chemical substances such as saponins, alkaloids, terpenoids and flavones with high percents. The herb is used for treating different human diseases and exploited for other traditional purposes in many African countries. The current phytochemical results of this plant proved what have been obtained by Frohne and Pfinder (1984) and Sujatha *et al*. (1998), who reported that *Artemisia annua* contains flavonoids, triterpenoids, volatile oil, alkaloids, tannins, saponins and sterols, with remarkable effects against some insects. Sharma *et al*. (1990; 2006) noticed that *Artemisia annua* contains a strong volatile oil which repels insects, and that alcohol extract of the plant was highly toxic when used against *Culex* larvae.

The phytochemical results of *Nerium oleander* were in conformity with Harbone (1984) and Goetz *et al.* (1998) who pointed this species as one of the most poisonous plants which contain numerous toxic compounds such as alkaloids and triterpenes. The plant is widely grown in Sudan, but is not consumed by animals due to its high toxicity and repellent actions. Such toxic effect in *N. oleander* was attributed mainly to the occurrence of cardiac glycosides in the plant sap (Desui, 2000). It was thought that this species may contain many other unknown or un-researched compounds with dangerous effects (INCHEM, 2005). The leaves and flowers of *N. oleander* showed high larvicidal effects against *Anopheles arabiensis* (Kehail and Bashir 2004; INCHEM, 2005).

TABLE 1. Phytochemical constituents of water extracts of different botanical parts of three plant species, during August 2009.

Plant species	Part	Chemical groups							
		Am	Sa	Al	Fl	Fn	Tn	St	Tr
Acacia nilotica	Leaves	-	+	+	-	+	-	-	-
	Fruits	+	+	+	-	+	+	-	+
Artemisia annua	Leaves	-	+	+	-	+	-	-	-
	Whole	-	+	-	-	+	-	-	-
Nerium oleander	Leaves	-	+	+	-	+	-	-	-
	Flowers	+	+	+	-	+	+	-	-

(+) present, (-) non present.
Amino acids (Am), saponins (Sa), alkaloids (Al), flavonoids (Fl), flavones (Fn), tannins (Tn), sterols (St) and triterpenes (Tr).

TABLE 2. Phytochemical constituents of ethanol extracts of different botanical parts of three plant species, during August 2009.

Plant species	Part	Chemical groups							
		Am	Sa	Al	Fl	Fn	Tn	St	Tr
Acacia nilotica	Leaves	-	-	+	-	-	-	-	-
	Fruits	+	-	-	+	+	+	+	-
Artemisia annua	Leaves	-	-	-	+	-	-	+	+
	Whole	-	-	-	-	-	-	-	+
Nerium oleander	Leaves	-	+	+	-	-	-	+	-
	Flowers	+	+	+	-	+	+	-	+

(+) present, (-) non present
Amino-acids (Am), saponins (Sa), alkaloids (Al), flavonoids (Fl), flavones (Fn), tannins (Tn), sterols (St) and triterpenes (Tr).

TABLE 3. Phytochemical constituents of petroleum ether extracts of different botanical parts of three plant species, during August 2009.

Plant species	Part	Chemical groups Am	Sa	Al	Fl	Fn	Tn	St	Tr
Acacia nilotica	Leaves	-	-	-	-	-	-	-	+
	Fruits	+	-	-	+	+	+	+	+
Artemisia annua	Leaves	-	-	-	-	-	-	+	+
	Whole	-	-	-	-	-	-	+	+
Nerium oleander	Leaves	-	-	-	-	-	-	+	+
	Flowers	+	-	-	-	-	-	+	+

(+) present, (-) non present
Amino-acids (Am), saponins (Sa), alkaloids (Al), flavonoids (Fl), flavones (Fn), tannins (Tn), sterols (St) and triterpenes (Tr).

CONCLUSION

The results of the current phytochemical screenings verified the richness of indigenous flora with potentially active botanical species which contain diversified groups of secondary metabolites. These neglected resources awaiting urgent attention for the sake of sustainable development. However, the three studied plants may represent good sources of various compounds such as alkaloids, saponins, flavones, triterpenoids and steroids. Moreover, *Acacia nitotica* can be considered as a unique rich source of tannins for industrial uses. The completion of research on these plants might lead to their commercial exploitation for various purposes including the production of biopesticides.

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