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Phytochemical constituents of different extracts from the leaves of *Chromolaenaodorata* (L.) King and Robinson

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ABSTRACT

Chromolaena odorata were screened for phytochemical constituents and fluorescence analysis of different extracts Petroleum ether,Chloroform, Methanolic and Aqueous extracts. The extract recovery percentage and pH of different solvent extracts of leaves of *Chromolaena odarata*.Methanolic extract showed highest yield of 17.8% and pH range of 6.05. Alkaloids, flavonoids, phenolics, tannins, steroids, saponins ,cardiac glycosides and carbohydrates were present in Methanolic extract and aqueous extract whereas in petroleum ether and chloroform extract alkaloids and flavonoids only present. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases.

Key words: *Chromolaena odorata*, phytochemicals, Fluorescence Analysis, medicinal plants

INTRODUCTION

Chromolaena odorata (L) King and Robinson Asteraceae commonly known as Siam weed, is a fast-growing perennial and invasive weed native to South and Central America. It has been introduced into the tropical regions of Asia, Africa and other parts of the world. It is an aggressive competitor that occupies different types of lands where it forms dense strands that prevents the establishment of other flora. It is a menace in plantations and other ecosystems. It suppresses young plantations, agricultural crops and smothers vegetation as it

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possesses allelopathic potentialities and growth inhibitors (Ambikaand Jayachandra, 1980; Ambika and Jayachandra, 1982; Muniappan and Marutani, 1988). The economic value of *C. odorata* is low. Consequently, there is a relative paucity of research works on it. It is a perennial shrub native of South and Central America. In recent decades, it has become a serious pest in the humid tropics of South East Asia, Africa and Pacific Islands. It spreads rapidly in lands used for forestry, pasture and plantation crops such as rubber, coffee, coconut, cocoa and cashew. It has been reported to have antispasmodic, aniprotozoal, antitrypanosomal, antibacterial and antihypertensive activeties. It has also been reported to possess anti-inflammatory, astringent, diuretic and hepatotropic activities (Watt and Brandwijk, 1962; Feng et al, 1964; Weniger and Robinean, 1988; Iwu, 1993). In the southern part of Nigeria, the leaves are used for wound dressing, skin infection and to stop bleeding. Some specific phenolic compounds have been isolated from the plant (Metwally and Ekejuba,1981).

The medicinal values of plants lie in their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body (Hill, 1952). A systematic search for useful bioactivities from medicinal plants is now considered to be a rational approach in nutraceutical and drug research. Therefore, the present work has been designed to evaluate the phytoconstituents, yield and pH of *C. odorata* with a view to contributing to the search for beneficial uses of this invasive plant which is a menace to farmers.

MATERIALS AND METHODS

Solvent extraction

50 g of air dried, coarsely powdered sample was successively extracted with different solvents in the increasing order of polarity (petroleum ether, chloroform, and ethanol) using soxhlet apparatus. Each time, before extracting with the next solvent, the powdered material was dried in hot air oven at 40°C. Finally, the material was macerated using hot water with occasional stirring for 16 h and the water extract filtered. The different solvent extracts were concentrated to dryness under reduced pressure using rotary vacuum evaporator, and weighed. The percentage yield (recovery) of evaporated extract was calculated as follows:

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$$\text{Yield (\%)} = \frac{[\text{Extract + container (g)}] - [\text{Empty container (g)}]}{\text{Sample weight (g)}} \times 100$$

The percentage yields were expressed in terms of the air dried drug.

Qualitative analysis

Phytochemical screening was carried out following the methods of Horbone, 1984 and Kokate *et al*., 1995.

Test for alkaloids
Wagner's test:
To 1 mL of the extract, a few drops of Wagner's reagent were added and the formation of a reddish brown precipitate indicates the presence of alkaloids.

Test for Flavanoids
Shinoda Test:
To 1 mL of the extract, magnesium turnings and 1-2 drops of concentrated hydrochloric acid were added. Formation of pink color indicates the presence of Flavanoids

Lead acetate test:
To 1 mL of the extract, few drops of 10% Lead acetate solution were added. Appearance of yellow colour precipitate indicates the presence of flavonoids.

Test for Phenols and Tannins
Lead acetate test:
To 1 mL of the extract, few ml of 1% lead acetate solution was added and the formation of precipitate indicates the presence of tannins and phenolic compounds.

Ferric chloride test:
To 1 mL of the extract, few ml of 5% ferric chloride was added. The development of dark bluish black color indicates the presence of tannins.

Sodium hydroxide test:
A small quantity of extract was dissolved in 0.5ml of 20%sulphuric acid solution. Followed by addition of few drops of aqueous sodium hydroxide solution, it turns blue presence of phenol

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Test for steroids and sterols
Salkowski's test:
The extract was dissolved in 2ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterols compound, in the extract.

Test for Saponins
Honey comb test:
5 ml of the extract was taken in a test tube and few drops of 5% sodium bicarbonate solution were added. The mixture was shaken vigorously and kept for 3 minutes .Formation of honey comb like froth shows the presence of saponins.

Foam test:
About 1 ml of the extract was diluted with 20ml distilled water and shaken well in a graduated cylinder for 15min. The formation of foam to a length of 1cm indicated the presence of Saponins and steroids.

Test for Glycosides
Legal test: The extract was dissolved in pyridine and freshly prepared sodium nitroprusside solution was added. The formation of pink to red color indicates the presence of glycosides.

Test for Protein & amino acids
Biuret test:
To 1 ml of extract, equal volume of 40% NaOH solution and two drops of 1% Copper sulphate solution were added. The appearance of violet colour indicates the presence of protein.

Ninhydrin test:
To 1 ml of extract, 2drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

Test for Carbohydrates
Fehling's test:
Five ml of Fehling's solution was added to 2 mL of the extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing power.

Benedict's test:
Five ml of Benedict's solution was added to 2 mL of the extract boiled in water bath. The appearance of red or yellow or green precipitate indicates the presence of reducing sugars.

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Fluorescence Analysis

A small quantity of dried and finely powdered leaves sample was placed on a grease free microscopic slide and added 1-2 drops of freshly prepared solution, mixed by gentle tilting the slide and waited for 1-2 minutes. Then the slide was placed inside the UV viewer chamber and viewed in day light, short (254 nm) and long (365 nm) ultraviolet radiations.
The colors observed by application of different reagents in various radiations were recorded [Gupta *et al*., 2006 and Kokashi *et al*.,1958].

RESULTS AND DISCUSSION

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds (P.Varadarajan *et al.*,2008). Table1 showed the extract recovery percentage and Ph of different solvent extracts of leaves of *Chromolaena odarata*.Methanolic extract showed highest yield of 17.8% and pH range of 6.05.Aqueous ,chloroform, petroleum ether extracts showed 13.8%,6.8%,6.6% Yield and pH range of 6.31,4.30,4.02 respectively.

Table 1 Extract recovery percentage and pH of different solvent extracts

S.No	Solvent	Yield%	pH
1	Petroleum ether	6.6	4.30
2	Chloroform	6.8	4.02
3	Methanol	17.8	6.05
4	Water	13.8	6.31

The phytochemical screening in the present study, has revealed the presence of Alkaloids, flavonoids, phenolics, tannins, steroids, saponins ,cardiac glycosides and carbohydrates were present in Methanolic extract and aqueous extract whereas in petroleum ether and chloroform extract alkaloids and flavonoids only present. CardiacGlycosides and protein were absent in all the three extracts except Methanolic extracts. Phenolics, alkaloids, and cardiac glycosides detected in the extracts are compounds that have been documented to possess medicinal properties and health-promoting effects (Salah *et al*., 1995; Del-Rio *et al*., 1997; Okwu, 2004; Liu, 2004). The flavonoids and phenolic compounds in plant have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti inflammatory, anti carcinogenic etc. (Lalitha *et al*., 2012). Saponins have hypotensive and cardiodepressant properties (Olaleye 2007) . Glycosides are naturally cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythmia (Brian *et al*.,1985)

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Table 2:**Preliminary phytochemical screening of different solvent extracts**

Phytoconstituents	**Test**	**Petroleum ether**	**Chloroform**	**Methanol**	**Water**
Alkaloids	Wagners test	+	+	+	+
Flavonoids	Shinoda Lead acetate	- -	- -	+ +	- +
Phenolics & tannins	Ferric chloride Lead acetate Sodium hydroxide	+ - -	+ - -	- + -	- + -
Steroids & sterols	Salkowski's test	-	-	+	+
Saponins	Honey comb Foam test	- -	- -	+ +	+ +
Cardiac Glycosides	Glycoside test	-	-	+	-
Protein	Biuret test Ninhydrin test	- -	- -	- -	- -
Carbohydrate	Fehlings test Benedicts test	- -	- -	- +	- +

Table 3: shows fluorescence analysis of powdered leaves of *Chromolaena odorata*

Powdered drug	**Visible/Day light**	**UV 254 nm (short)**	**UV 365 nm (long)**
Untreated leaf powder	Dark green	Dark green	Dark green
Treated with methanol	Dark green	Dark green	Dark green
Treated with1% glacial acetic acid	Deep greenish brown	Greenish brown	Green
Treated with 10% NaOH	Dark green	Dark green	Green
Treated with dilNH_3	Green	Green	Light green

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Treated with concNH$_3$	Brown	brown	Light brown
Treated with 1MH$_2$SO$_4$	Green	Green	Light green
Treated with 1M HCL	Green	Green	Light green

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation (Ansari 2006).

Conclusion

The presence of phytoconstituents make the plant useful for treating different ailments and have a potential of providing useful drugs of human use. In the present study, we have found that most of the biologically active phytochemicals were present in the Methanolic and aqueous extracts of leaves of *Chromolaena odarata*. Since the Methanolic extract contains more constituents it can be considered beneficial for further investigation.

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