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PRELIMINARY PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIOXIDANT ACTIVITY OF *DATURA STRAMONIUM* L. COLLECTED FROM JIMMA, SOUTH WEST ETHIOPIA

KUMAR GANESAN[1], SURESH KUMAR P. NAIR[1], HENOK GULILAT AZALEWOR[1], NEETHU LETHA[2] AND SHARMILA BANU GANI[3*]

[1]Department of Biomedical Sciences, School of Public Health and Medical Sciences, Jimma University, Jimma 378, Ethiopia
[2]Department of Zoology, Government Women's College, Trivandrum, Kerala, India
[3]Department of Zoology, NKR Government Arts College for Women, Namakkal-637001, Tamilnadu, India

ABSTRACT

The plant derived materials come into use in the contemporary medicine through aboriginal cure in traditional systems of medicine. Medicinal plants are chief antidote for numerous diseases and have been used since time immemorial. The present study was investigated to identify the preliminary phytochemical analysis of crude extracts of leaves, fruits and stem of *Datura stramonium* using aqueous, benzene & CCl_4 as solvents and *in vitro* antioxidant activity of dry and fresh leaves of *D. stramonium* L. by using DPPH methods. The extracts were subjected to qualitative phytochemical screening using standard procedures. Three different extracts of leaves, stem and fruits of *D. stramonium* were found to contain various secondary metabolites like Triterpenoids, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids and Tannins. The results of antioxidant properties of both fresh and dry extracts exemplified that aqueous has more potent free radical scavenging activity than the organic solvents; the absorbance of the prepared samples was measured using UV- visible spectroscopy at 520 nm wavelength. The phytochemicals generated data from the three crude extracts of *D. stramonium* may be used as tools for quality control of drugs in the future, for the healing of a diversity of disease conditions.

KEY WORDS: ***D.stramonium,*** **preliminary phytochemical analysis, secondary metabolites, antioxidant activity.**

KUMAR GANESAN
Department of Biomedical Sciences, School of Public Health and Medical Sciences, Jimma University, Jimma 378, Ethiopia

INTRODUCTION

Medicinal herbs play a foremost role in the treatment of human strain and diseases globally. The requirement of herbs is greater than ever in worldwide due to growing identification of natural product. Herbal medicine is a vital part of both conventional and contemporary system of medicines[1]. Medicinal plants contain a numerous primary and secondary metabolites and those compounds are used to heal infectious and chronic diseases. Furthermore, it is the traditional form of health care known to civilization and it lacks documentation in the lights of contemporary medicine. WHO provide a statement that more than 80% of the world's population needs on conventional medicine for their principal healthcare[2]. (*Family: Solanaceae*) is a widespread flowering plant. It is one of the extensively well recognized traditional medicinal herbs (Figure-1). The plant is inhabitant to Asia, but now become grow wild in many other regions in Ethiopia, Eritrea, tropical Africa, West Indies and the United States. About twelve species of *Datura* distributes over tropical and hot temperate regions of the earth. *Datura* is folklore medicinal plant and as known as hallucinogenic drug worldwide. The plant was used internally to treat depression, convulsion and mental illness. Externally it uses as an ointment for burns, inflammation and rheumatism[3]. *Datura* is also widely used as insects repellent[4] and healing of Parkinsonism and hemorrhoids. It has extensive pharmacological properties of antiasthmatic activity[5], antimicrobial Activity[6, 7], antifungal activity[8], vibriocidal activity[9], anticancer activity[10], anti-inflammatory activity[11], anticholinergic activity[12, 13], acaricidal, repellent and oviposition deterrent properties[14], and larvicidal and mosquito repellent activities[15]. In spite of a number of tasks on pharmacological and pharmacognosy activity were done in different places of the world; however the medicinal relevance of the phytoconstituents of this herb is still inadequate in many of earlier reports. The preliminary phytochemical screening test in five different solvents namely ethanol, hexane, chloroform, petroleum ether and acetone of leaves crude extract of D. stramonium and its antimicrobial activities were done by Solomon Girmay[16]. This is therefore, the main objectives of the present study was planned to investigate the preliminary phytochemical analysis of leaves, stem and fruits of *D.stramonium* L. using aqueous, benzene and CCl_4 crude extracts. In addition the study planned to extent invitro antioxidant activity of dry and fresh leaves of *D.stramonium* L. collected from Jimma, south west Ethiopia.

Figure 1
1 The leaves, stem and fruits of D.stramonium L.

MATERIALS AND METHODS

Chemicals

DPPH (2, 2-diphenyl-1-picrylhydrazyl) was procured from Sigma-Aldrich, USA. All the chemicals used in this experiment were of analytical grade.

Collection of Plant sample

D.stramonium L. was collected from Roadside, near Jimma University, Jimma, South West Ethiopia. The plant was authenticated by Jimma University Taxonomist Dr. Ramesh Moochikkal and comparison with reference specimens preserved at the Jimma University Botanical Science and Herbarium. Voucher Herbarium specimens are kept in the Herbarium for future references. The whole plants were washed with tap water then surface sterilized in 10% sodium hypochlorite solution to avoid the stain of any microbes, and then rinsed with sterile distilled water.

Preparation of Plant Materials

Leaves, fruits and stem of *D.stramonium* were dried for 12hrs in a hot air oven at 50^0 C. The dried materials were ground using an electric blender to obtain a fine powder. The powder was additionally passed through a

2mm filter to get fine particles. The powdered samples were stored in separate fresh glassware container until required for analysis.

Preparation of extracts

Aqueous, Benzene, and CCl_4 extracts of leaves, fruits and stem of D*. stramonium* were prepared in 10g/ 100 ml. The solvents of organic extracts were dried at 60^0 C protected from light and the organic solvents were evaporated using a rotary evaporator under pressure for 45 min resulting in a solid crude extract. These extracts were used for the investigating of preliminary phytochemical analysis and *in vitro* antioxidant activity.

Preliminary phytochemicals screening

Test for alkaloids

About two ml of the leaves extract was added to 2 ml of hydrochloric acid. To this acidic medium, 1 ml of Dragendroff's reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloids.

Test for amino acids

About one ml of the extract was treated with a few drops of Ninhydrin reagent. Appearance of purple colour shows the presence of amino acids.

Test for anthraquinones

About five ml of the extract solution was hydrolysed with diluted Conc. H_2SO_4 extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones. About one ml of the extract, a few drops of dilute sodium hydroxide was added. An intense yellow colour was produced in the plant extract, which become colourless on the addition of a few drops of dilute acid indicates the presence of flavonoids.

Test for glycosides

The extract was hydrolysed with hydrochloric acid for few hours on a water bath. To the hydrolysate, 1ml of pyridine was added and a few drops of sodium nitroprusside solutions were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

Test for saponins

The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam showed the presence of saponins.

Test for steroids

About one ml of the extracts was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for tannins

About two ml of the extract and a few drops of 1% lead acetate were added. A yellow precipitate was formed, indicates the presence of tannins.

Test for triterpenoids

About two ml of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of Conc.H_2SO_4. Formation of reddish violet colour indicates the presence of triterpenoids.

Antioxidant activity

The assessment of free radical scavenging activity of dry and fresh crude leaves extracts of D.stramonium was described by Alabri et al.[17] with small alteration. The various dry and fresh crude leaves extracts of *D. stramonium* at various concentrations (12.5, 25, 50, 100 and 200 ppm, respectively) were taken in separate test tubes. One ml of DPPH solution and 1ml of methanol were added in each to test tube and shaken vigorously and allowed to stand at room temperature in a dark place for 1hr. The blank and positive controls were prepared as the same like as test without any plant extract. Ascorbic acid was used as standard (50 ppm). The absorbances of the samples were measured using UV- visible spectroscopy at 520 nm wavelength. Each method in this experiment was replicated three times. Free radical scavenging activity of the tested plant crude extract samples were estimated as an inhibition percentage and were calculated by using the following formula,

Measurement of radical scavenging activity (%)

$$\% \text{ Inhibition} = \frac{A_{control} - A_{extract}}{A_{control}} \times 100$$

RESULTS

Preliminary phytochemical analysis

In the present study, preliminary phytochemical screening has been done in leaves, fruits and stem crude extracts of *D.stramonium* L. using aqueous, benzene and CCl_4 showed the presence of phytochemical constituents namely alkaloids, flavonoids, glycosides, saponins, *anthraquinones*, steroids, tannins, triterpenoid, and aminoacids shown in Table I.

Table I
Preliminary phytochemical analysis in aqueous, benzene, CCl_4 extracts of Datura stramonium L.

Analysis	Leaves			Fruit			Stem		
	Aqueous	Benzene	CCl_4	Aqueous	Benzene	CCl_4	Aqueous	Benzene	CCl_4
Wagner's Test	+	+	+	+	+	+	+	+	+
Ninhydrin	-	-	-	+	-	-	-	-	-
anthraquinones	-	+	+	-	-	-	+	-	-
Flavonoids	-	-	-	-	-	-	-	-	-
Glycosides	-	+	+	+	+	+	+	+	+
Saponins	+	+	+	-	-	-	-	+	-
Steroids	+	+	+	-	-	-	-	-	-
Tannins	-	+	+	-	-	-	-	-	-
Triterpenoids	-	+	+	-	-	-	-	-	+

+ = Presence; - = Absence

Antioxidant capacity

The antioxidant activity of aqueous, benzene and CCl_4 crude extracts from the dry and fresh leaves of *D. stramonium* at different concentrations (12.5, 25, 50, 100 and 200 ppm) showed activity ranging from 30–60% for dry samples and 40–70% for fresh samples. The absorbance was increased in aqueous crude extract compare to concentrations of organic crude extracts from dry and fresh samples (Figure 2 and Figure 3).

Figure 2
Antioxidant capacity of aqueous, benzene, CCl_4 crude extracts from dry leaves of* D. *stramonium

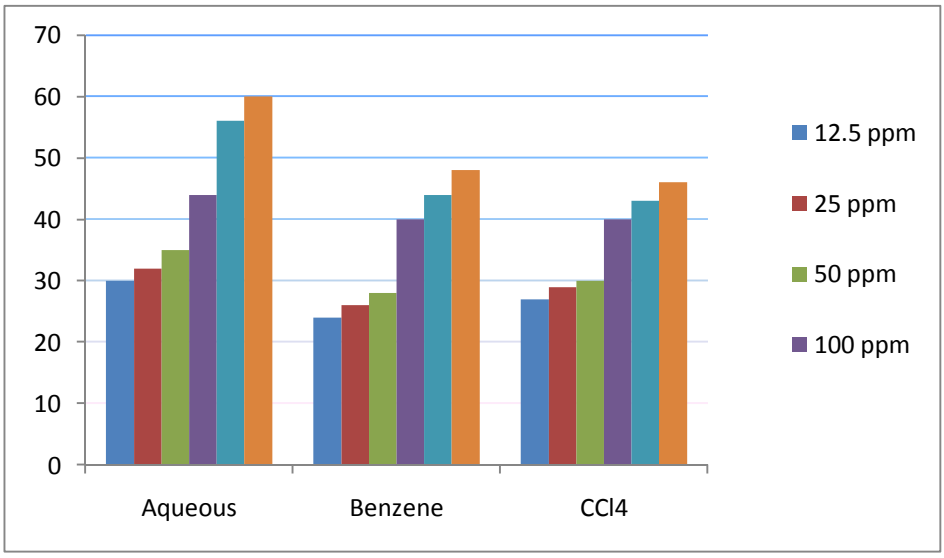
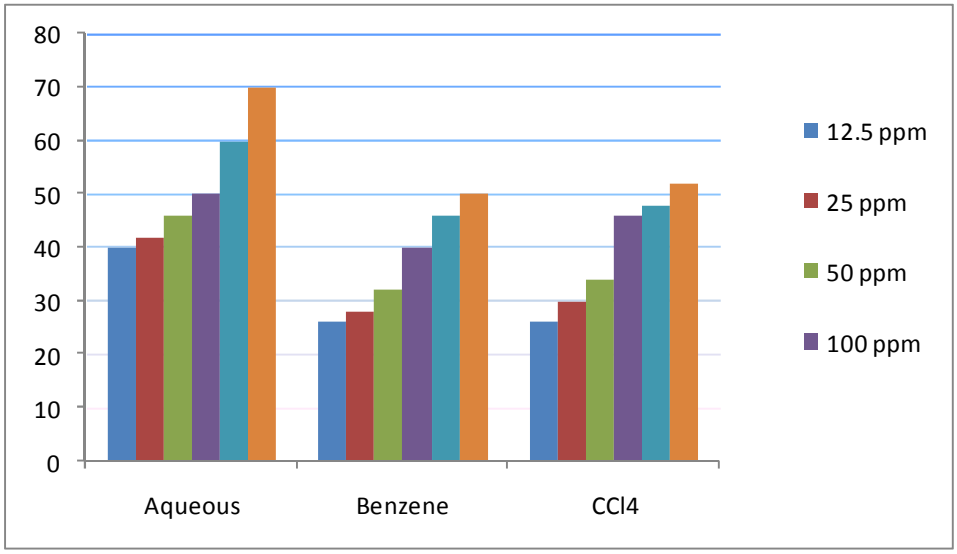


Figure 3
Antioxidant capacity of aqueous, benzene, CCl_4 crude extracts from fresh leaves of* D. *stramonium



DISCUSSION

The initial phytochemical screening tests may be helpful in the screening of the bioactive compounds and eventually may help to detection and development of new drugs. Further, these tests make easy their qualitative separation and quantification of pharmacologically dynamic compounds[18]. The phytochemical screening in the present study has publicized the presence of alkaloids, tannins, anthraquinones, steroids, glycosides, saponins and triterpenoids in the leaves extract. Further the presence of different phytochemicals in the three different solvent extracts may be responsible for the therapeutic properties of *D. stramonium*. Tannins are phenolic compounds that are acting as principal antioxidants or free radical scavengers. Since these phenolic compounds were originated to be present in the extracts, it might be accountable for the potent antioxidant capacity of *D. stramonium*. These phytochemicals of medicinal plants have primarily reported for their medicinal value, which can be valuable for therapeutic index. For instance, saponins and glycosides proved as hypotensive and cardiodepressant properties[19], which are helpful for the treatment of congestive heart failure and coronary artery diseases[20]. The occurrence of saponins in aqueous, benzene and CCl_4 extracts and glycosides presence in benzene and CCl_4 extracts of leaves of *D. stramonium* might play a role in the cardioprotective potential. Tannins and alkaloids have the potential of anti-hyperglycaemic and anti- inflammatory activities[21]. Moreover, the terpenoids have also been revealed to diminish the blood sugar level in animal models[22]. In addition, the steroids and triterpenoids demonstrated the analgesic properties and central nervous system activities[22-24]. Hence the initial phytochemical studies are helpful in finding chemical constituents in the plant material that may help to their identification and quantification of pharmacologically active principle. The antioxidant potential of three solvent extracts from fresh and dry leaves of *D. stramonium* at various concentrations was determined and compared by DPPH method. The principle of antioxidant activity is dealing with oxidative free radicals and makes them as stable. The responsibility of DPPH method is also acting that the antioxidants react with the stable free radical, and DPPH is converted into 2,2-diphenyl-β-picrylhydrazine to produce a new colour. The rate of colour changes progressively reduced to indicate the free radical scavenging potentials. The aqueous crude extracts of *D. stramonium* contain alkaloids, saponins, tannins, glycosides and aromatic compounds. All these secondary metabolites were able to change the colour in DPPH solution by their hydrogen donating ability[25-27]. From the results it appears that the three crude extracts from the dry and fresh leaves of *D. stramonium* have hydrogen donating capacity and it is acting as a free radical scavenger. The results of antioxidant potentials of dry leaves of *D. stramonium* were set up in the sequence of aqueous extract > benzene > CCl_4 extract. The free radical scavenging activity for all solvent extracts from fresh samples was superior rather than dry samples. The antioxidant functional difference between dry and fresh crude extract samples might perhaps due to the extraction methods, samples dispensation or aeration. During the dispensation, some active unstable compounds may have been shattered or disappear from the dry samples. This may be given functional different antioxidant activity among dry crude extracts and fresh samples.

CONCLUSION

The results of phytochemical analysis showed the leaves, fruit and stem extracts of *D. stramonium* indicates their potential as a source of bioactive principles that may supply drugs for modern medicines. Further studies are therefore required to validate their antimicrobial, antihyperglycemic, anti-inflammatory, and antihelminthic activities. In addition, extraction, cleansing and categorization of the active principles are required to make the plant has novel interesting studies.

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