Volume-3, Issue-7, July-2014 • ISSN No 2277 - 8160

Research Paper Zoology

Phytochemical Screening of the Leaves of Cleome Gynandra LINN (Cleomaceae)

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ABSTRACT

Phytochemical screening has opened a new area of research in biological science. It is proven that phytochemicals provide human beings with treatment of various diseases. Phytochemical techniques have played a significant role in searching raw materials and resources for pharmaceutical industry. Preliminary phytochemical tests are helpful in finding and locating chemical constituents which are source of pharmacologically active principles. During the present study, the medicinally important plant i.e. Cleome gynandra, was investigated for its phytochemical constituents. The methanolic extract of the plant was used and tested for alkaloids, phenols, flavonoids, saponins, tannins, steroids and protein. Cleome gynandra showed absence of alkaloid except all the phytochemical mentioned above. Moreover Cleome gynandra leaf extract showed strong reducing power. In the DPPH radical scavenging assay, the percentage yields were found to be 0.78, 2.34, 4.46% for concentrations viz. 10µg, 50µg and 100µg respectively. The phytochemical study was performed by using standard phytochemical methods.

KEYWORDS : Cleome gynandra, phytochemical screening, phenols, tannins, flavonoids

INTRODUCTION:

The use of local plants in folk medicinal practices has a long history. During the past decade, traditional systems of medicine have become a topic of global importance. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) of have always maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs. The resource base of the traditional medicinal practices prevalent in rural and tribal villages of India and abroad is mainly the plants[1]. Herbal medicines have been recognized as a valuable and readily available resource for primary health care and; WHO has endorsed their safe and effective use (WHO, 1993). It has been estimated that even today, 80% of the world population rely on traditional herbal medicine for their primary health care (Absar A. Qureshi *et al.,* 2008). Although the efficacy and mechanism of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their chemical constituents (Park and Puzzutto, 2002). The screening of plant extracts of plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes[2]. The leaves possess antinociceptive, anti-inflammatory and antipyretic activities[3].

Reactive oxygen species [ROS] have been implicated in many diseases like cancer, diabetes, atherosclerosis and heart disease [4, 5]. ROS can be classified into free radicals [superoxide ion] (O^{2-}), hydroxyl radicals (OHO) and non free radicals (hydrogen peroxide) (H_2O_2) [5, 6, 7]. Antioxidants have the ability to stabilize these free radicals and revive the cells from the damage caused by free radicals. The DPPH antioxidant assay is based on the ability of 1-1-diphenyl-2- picrylhydrazyl, is a stable free radical which get decolorized in the presence of antioxidants. The DPPH free radical contains an odd electron, which is responsible for the absorbance at 517nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance.

Synthetic antioxidants like butylated hydroxytoluene and buytlated hydroxyanisole, commonly used in processed foods, possessed some side effects that have limited their use as antioxidant agents [6,711, 12]. In the present work, qualitative phytochemical analysis and free radical scavenging activity have been carried out in Cleome gynandra of Kamup district.

Materials and methods

Collection of plant materials

Fresh leaves of *Cleome gynandra* was collected from different parts of Kamrup district. They in fresh condition were washed under running tap water and then again with distilled water. The plant materials were air dried in shade for 5 days except seeds and then homogenized to fine powder and stored in airtight bottles with proper labeling.

Preparation of extracts

Powdered plant materials were collected and weighed carefully. 50g of each of the plant material was weighed and soaked in 300 ml of methanol. The mixtures were kept in shaker for 48 hours and filtered. The filtrates were kept in rotary evaporator in low temperature under reduced pressure till dryness. Extracts thus obtained were examined chemically and screened for phytochemical screening. The extract was kept in refrigerator when not in use.

Phytochemical analysis

In present study phytochemical tests were carried out and the presence of various chemical constituents in the studied plant extracts was determined by preliminary phytochemical screening. The tests were done for the presence of the active chemical constituents such as alkaloids, steroids, flavonoids, saponins, tannin, phenols and proteins by the following procedure.

Alkaloids

The methanolic extracts were heated with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation. (Siddiqui and Ali, 1997). Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Steroids

20mg of extracts were added with 2.5 ml of acetic anhydride and 2.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids. (Siddiqui and Ali, 1997)

Flavonoids

20mg of extracts were mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids. (Siddiqui and Ali, 1997)

Saponins

15 mg of extracts were mixed with 5ml of distilled water in a test tube

and it was shaken vigorously. The formation of stable frothing were Observed. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion. (Siddiqui and Ali, 1997)

Tanins
To 0.5 ml of extract solution 1ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins (Iyengar, 1995).

Phenols
Crude extract was mixed with 2ml of 2% solution of ferric chloride. A blue-green or black coloration indicated the presence of phenols. (Siddiqui and Ali, 1997)

Proteins
To 2 ml of the extract solution 1ml of 40% sodium hydroxide was added. To it 1-2 drops of 1% copper sulphate solution was added. A violet colour indicates presence of proteins. (Siddiqui and Ali, 1997)

Free-radical scavenging activity
Different concentrations (10µg, 50µg and 100µg) of the test sample and Butylated hydroxy anisole (BHA) were taken in different test tubes. The volume was adjusted to 500µl by adding Methanol. Five milliliters of a 0.1 mM methanolic solution of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) was added to these tubes and shaken vigorously. A control without the test compound, but with an equivalent amount of methanol was maintained. The tubes were allowed to stand at RT for 20 minutes. The absorbance of the samples was measured at 517 nm[8]. Free Radical scavenging activity was calculated using the following formula:

% radical scavenging activity =

$$\frac{\text{(control OD - sample OD)}}{\text{Control OD}} \times 100$$

Results and discussions
The phytochemical characteristics of the tested medicinal plant were summarized in the table-1. The results revealed the presence of medically active compounds in the plant studied. It was found that, the leaves of *Cleome gynandra* showed the presence of all the phytochemicals viz. proteins, phenols and tannins, flavonoids and saponins except alkaloids. The DPPH (1,1-dipheny-2-picrylhydrazyl) radical scavenging activity of *Cleome gynandra* is shown in Table.2. The methanolic extract of *Cleome gynandra* leaves showed percentage yields of 0.78, 2.34, 4.46% for the concentrations viz. 10 µg, 50 µg and 100 µg respectively.

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities[4]. Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, steroids and alkaloids. In the present study, qualitative tests for all the extracts showed significant indication about the presence of metabolites. These findings of phytochemicals in different plants were good enough to reflect its importance. The phytochemicals present in Cleome gynandra such as phenols and flavonoids are ubiquitous groups of metabolites that have different biological properties. Various studies reveal that plants showing the presence of phenolics also contain antioxidant properties[5,6]. Nowadays, steroids found in plants are of great importance for their relation with sex hormones. Natural antioxidants are playing a significant role in the metabolic system by scavenging the free radicals from body. From the study, it is found that *Cleome gynandra* is one of the plants which yield a high amount of antioxidants. People are now more interested in plants due to their effectiveness and for having no side effects[7].

Conclusion
Plants are the best source of chemical compounds having different biological properties that could make human life easier by treating various chronic ailments. Unlike the modern drugs that cause many side effects, by using plant derived chemicals many diseases can be cured without any side effects.

Table 1: Phytochemical constituents of medicinal plants studied:

Plant	Alkaloids	Steroids	Flavonoids	Saponins	Tanins	Phenols	Proteins
Cleome gynandra	–	+	+	+	+	+	+

+ = presence, - = absence

Table 2: Percentage Free radical scavenging activity

Conc.	Cleome gynanda	BHA
10µg	0.78	13.27
50 µg	2.34	50.72
100µg	4.46	71.24

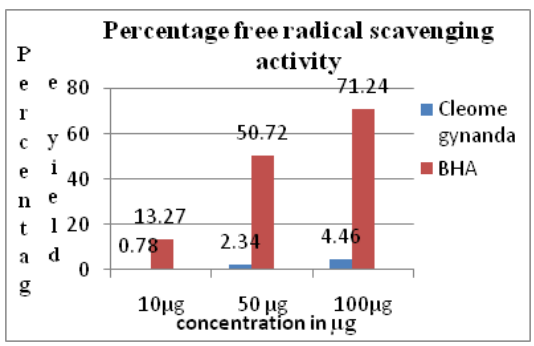

Fig 1: showing the percentage free radical scavenging activity

(i)

(ii)

Fig 2: showing (i) Leaf of Cleome gynandra, (ii) whole plant with flowers

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