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PHYTOCHEMICAL SCREENING, QUANTITATIVE ANALYSIS AND TRACE ELEMENTS IN ETHANOL, CHLOROFORM EXTRACT OF *RUELLIA PROSTRATA*

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ABSTRACT

Traditional medicines with therapeutic utility have been used since antiquity and are still contributing a significant role in the primary health-care system. Thus this article focused on the extraction, phytochemical, macro and micro minerals and antioxidant potential of *Rullia prostrate* ethanol and chloroform flower extract. The phytochemical screening revealed that the plant is a rich source of different secondary metabolites like flavonoids, phenol. Elemental analysis showed the presence macro and micro minerals. Hence the ethanol and chloroform flower extract of *Rullia prostrate* shows many compounds and may have been used in traditional medicine for prevention of several diseases.

KEYWORDS

Phytochemical, *Rullia prostrate* and Flavonoid contents.

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INTRODUCTION

Nature acts as an endless source of the medicinal entities, pharmacophores, novel chemophytes which contribute in the field of drug development for the betterment of the human illness since the ancient time. Medicinal plants have been used for thousands of years to cure various human diseases as the plants contain many constituents which have high therapeutic values. Plants have great importance due to their nutritive value and they are the major source of medicines which play an important role in the human history[1]. Plants synthesize primary metabolites (proteins, fats, nucleic acids and carbohydrates) by simple substances such as water,

carbon dioxide, nitrogen and a number of inorganic salts in small amounts. These primary metabolites are transformed into secondary metabolites (alkaloids, steroids, terpenoids, saponins, flavonoids etc.,) that are used as drugs[2]. Figure No.1 shows the *Ruelliaprostrata* (Acanthaceae) is a perennial herb, commonly found as a weed throughout India. Reviewing the previous work of the *Ruellia prostrata* was found to have very little chemical and biological studies. This plant is widely distributed throughout India from extending to east Africa, central and peninsular India[3]. Anti-oxidants from plant materials play a significant role in termination of these free radicals, thereby protecting the body from these diseases. Herb is mainly known for its traditional use as an anti-inflammatory and anti-cancer against the epidermis of nasopharynx region and possesses wound healing properties[4,5]. Arthritis is an inflammatory disorder which manifests by destructive arthropathy and extra-articular manifestations, leading to severe disability and premature mortality[6]. The present study aimed at screening the phytoconstituents and anti-oxidant potential of Kenyan *Ruellia prostrata* to make this plant useful for the formulation of analgesic and anti-arthritic drug for the management of arthritis. *Rule prostate* is an indigenous medicinal plant, which present in moist, shady places throughout India. It is widely distributed in Arica, Srilanka, Pakistan and throughout India[7]. The plant is commonly known as bell weed and black weed[8,9]. The objective of this study was therefore to investigate the flower extracts of the *Rullia prostrata* for antioxidant activities as well as to determine the phytochemical contents and macro and micro minerals.

MATERIAL AND METHODS

Plant material- Identification and authentication

Rullia prostrate flower was selectively removed from the plant in and around areas of Pudussery, Palakkad, Kerala and identified by a plant taxonomist. BSI/SRC/5/23/2022/Tech/96.

Preparation of *Rullia prostrate* flower extract

Rullia prostrate flower was washed, dried in a hot air oven at 40°C and subsequently ground into powder in an electric grinder. Delipidation was performed with ethanol and chloroform (60-80°C) for overnight. soxhalation was performed with 95% ethanol. Ethanol was evaporated in a rotary evaporator at 40-50°C under reduced pressure. The yield of the flower extract was around 13.5 % of dry weight.

Qualitative Phytochemical Screening

The plant extracts obtained by using ethanol and chloroform extraction process and it is subjected to different phytochemical tests to identify the plant constituents by using standard following methods[10,11].

Test for protein

The protein content of all the plant extracts was estimated following the method[12]. Values were expressed as µg protein/mg plant extract using the calibration curve of BSA.

Test for Carbohydrates

The presence of carbohydrates was confirmed when 2 ml of extract was treated with 1ml of Molisch's reagent and a few drops of concentrated sulfuric acid which resulted in the formation of purple or reddish color.

Test for Phenols

2ml of distilled water, followed by few drops of 10% ferric chloride was added to 1ml of the extract. Formation of blue or green color indicates presence of phenols.

Test for Tannins

To 1ml of extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for Flavonoids

To 2ml of extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

Test for Saponins

2ml of extract, 2ml of distilled water were added and shaken in a graduated cylinder for 15 min lengthwise. It resulted in the formation of 1cm layer of foam that indicated the presence of saponins.

Test for Glycosides

To 2ml of extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

Steroids

To 1ml of fruit extract equal volume of chloroform is added and a few drops of concentrated sulphuric acid added appearance of brown ring indicates the presence of steroids and appearance of the bluish brown ring indicates the presence of phytosteroids.

Test for Terpenoids

0.5ml of the extract was treated with 2ml of chloroform and conc. sulphuric acid. Formation of red brown color at the interface indicates the presence of terpenoids.

Test for Alkaloids

To 2ml of extract, 2ml of concentrated hydrochloric acid was added. Then a few drops of Mayer's reagent were added. The presence of green color or white precipitate indicates the presence of alkaloids.

Quantitative determination of secondary metabolites

Estimation of flavonoids

The total flavonoid content in the sample was estimated by the method of Chang. A volume of 0.25ml of the sample was diluted to 1.25ml with distilled water. 75µl of 5% sodium nitrite was added and six minutes 0.15ml of aluminium chloride solution was added. 0.5ml of 0.1M NaOH was added after 5 min and made up to 2.5ml with distilled water. The solution was mixed well and the absorbance was read at 510nm along with standard quercetin at concentration. The results are expressed as mg of flavonoids as quercetin equivalent / gm of dried sample.

Total Phenolic Content (TPC)

Total phenolic content of the extract was determined according to the Folin-Ciocalteau method of Slinkard and Singleton with some modifications. Briefly, 0.1ml of extract, 1.9ml distilled water and 1ml of Folin-Ciocalteau's reagent were seeded in a tube, and then 1ml of sodium carbonate was added. The reaction mixture was incubated at 25°C for 2 h and the absorbance of the mixture was read at 765nm. The sample was tested in triplicate and a calibration curve with six data points for catechol was obtained. The results were compared with the cortical calibration curve and the total phenolic content of the sample was expressed as mg of catechol equivalents per gram of extract.

Mineral concentration

Trace minerals, namely Cu, Co, Fe, and Zn were estimated in concentrate ethanol and chloroform extract of *Rullia prostrate* flowers by using an atomic absorption spectrophotometer (AAS 4141, ECIL-Elements, India) and macro- minerals like Na and K were measured by using flame photometer (Model no. 1381, ESPIO, Japan). All the results were expressed as µg mg-1 of extract.

Statistical analysis

All the assays were carried out in triplicate. Experimental results are expressed as mean ± standard deviation. The results were analyzed using one-way analysis of variance and the group means were compared using Duncan's multiple range tests using SPSS version 16.

RESULTS AND DISCUSSION

Phytochemicals are defined as bioactive non-nutrient plant compounds found in fruits, vegetables, grains and other plant foods that have been linked to reducing the risk of major chronic diseases[13]. Table No.1 shows the Medicinal values of plants i.e. their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds produce a definite physiological action on the human body[14]. Medicinal plants contain many antioxidants such as vitamin (A, C and K), carotenoids, flavonoids (flavones, isoflavones, flavonones, anthocyanidin, catechin and isocatechin), polyphenols (ellagic gallic acid and tannin).

Several reports say that these compounds possess remarkable antitumor, antidiabetic and antioxidant activity[15-17]. Figure No.2 and Figure No.3 shows the Flavonoids are a group of naturally occurring polyphenolic compounds primarily from fruits and vegetables. Several studies have evaluated the cyto-toxic effect of saponins against tumor development.

The active components in several herbal medicines that have been used as chemotherapeutic agents in Eastern countries were shown to be saponins. A Chinese herbal drug, 'Yunan Bai Yao' has been used as a hemostatic agent and it is known to promote wound healing[18].

The *Rullia prostrate* flower possess significant amount of vitamin (C, K), carotenoids and phenols. Zinc maintain various reactions of the body which help to construct and maintain DNA, required for the growth and repair of body tissues, important element of ligaments and tendons[19].

Deficiency is characterized by megaloblastic anemia, fatigue, weakness, constipation, loss of appetite and weight loss[20]. Neurological changes, such as numbness and tingling in the hands and feet, can also occur. Both the plants show minimum amount of elements and better nutritive value in the flowers (Figure No.4 and Figure No.5).

Table No.1: Showes the Phytochemical screening of *Rullia prostrate* flowers extract

S.No	Qualitative test	*Ruelliaprostrata* Flowers extract	
		Ethanol	Chloroform
1	Proteins	-	+
2	Carbohydrates	+	-
3	Phenols	-	+
4	Tannins	+	+
5	Flavonoids	+	-
6	Sapoins	-	-
7	Glycosides	+	+
8	Steroids	+	+
9	Terpenoids	-	-
10	Alkaloids	+	+

Figure No.1: *Rullia prostrate* wholeplant

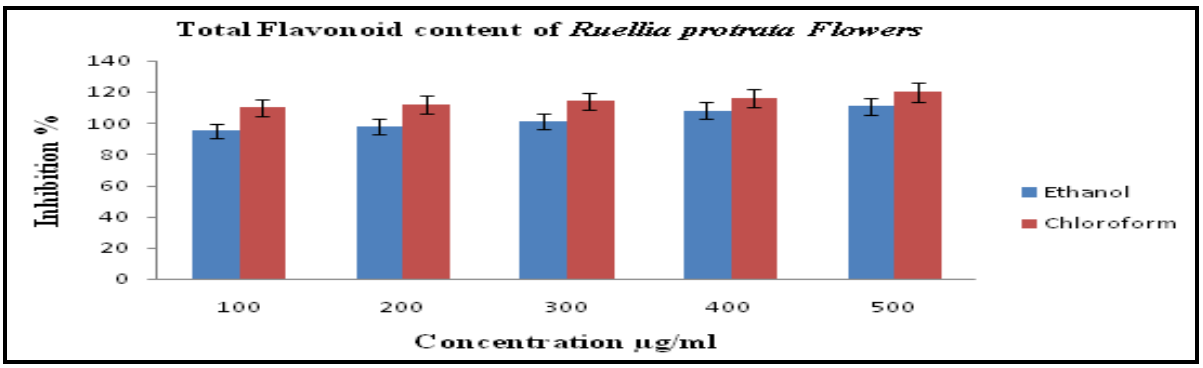

Figure No.2: Shows the total flavonoid content *Rullia prostrate* flower extract

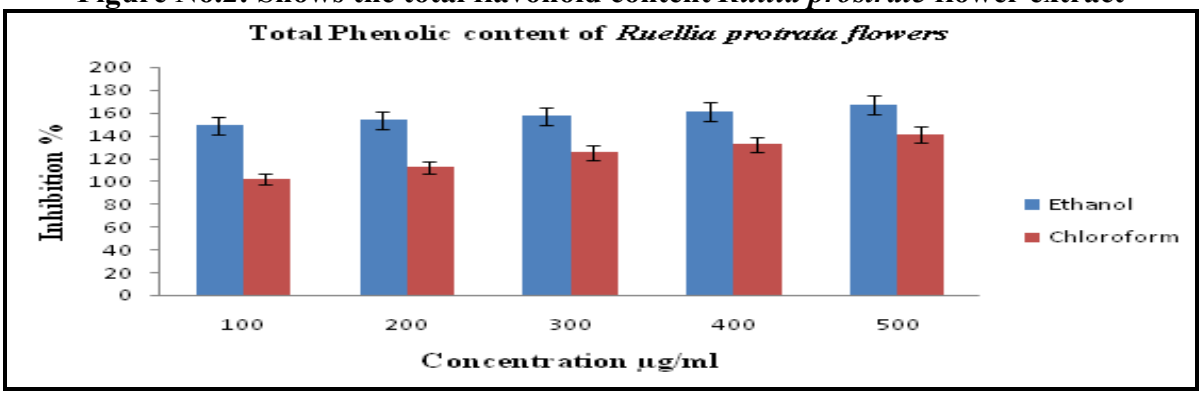

Figure No.3: Shows the total phenolic content *Rullia prostrate* flower extract

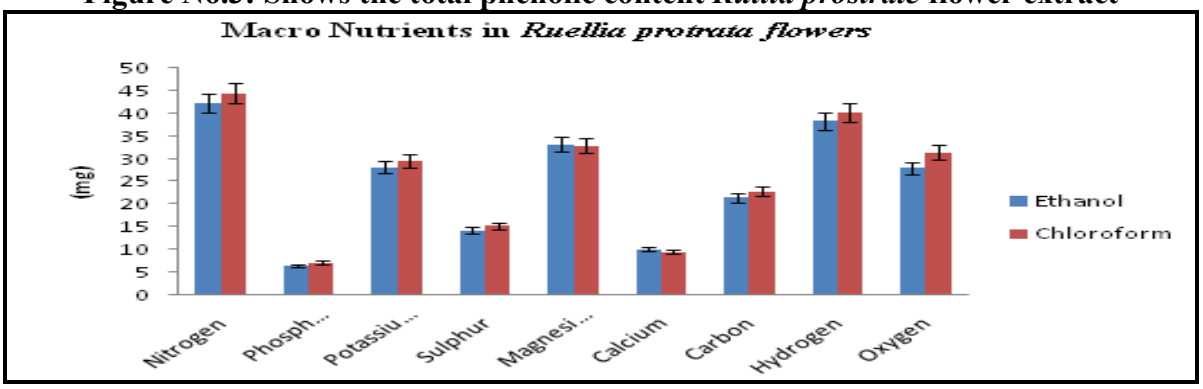

Figure No.4: Shows the Macro nutrients *Rullia prostrate* flower extract

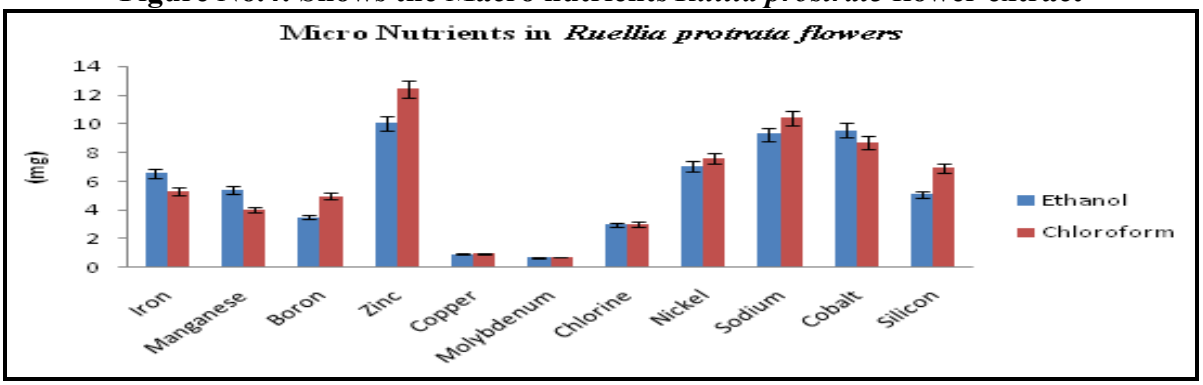

Figure No.5: Shows the Micro nutrients *Rullia prostrate* flower extract

CONCLUSION

Rullia prostrate flower is a significant traditional medicinal plant that is used extensively by tribes to cure a wide range of diseases. The study concluded that the plant is is a rich source of different secondary metabolites like flavonoids, saponins, carbohydrates, proteins phenol, glycosides, diterpenes and tannins. Quantitative analysis revealed that the plant shows a good amount of flavonoid and phenols. This study confirms that plant showed a great potential of anti oxidant activity. The Antioxidant studies on the *Rullia prostrate* flower have scientifically shown its rich antioxidant potentials which in addition to other factors could be helpful in validating the traditional uses of the plant in the treatment of several ailments.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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