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CROTON MACROSTACHYUS ROOTS USED TO STUDY PHYTOCHEMICAL SCREENING, TEST FOR INORGANIC ELEMENTS, PROXIMATE ANALYSIS, QUALITATIVE AND QUANTITATIVE CHARACTERIZATION OF PHYTOCONSTITUENTS, ANTIOXIDANT ACTIVITIES AND FREE RADICAL SCAVENGING ACTIVITY

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ABSTRACT

The present study was to evaluate preliminary photochemical analysis, inorganic elements, antioxidant activities, proximate analysis and free radical scavenging activity of root extracts of *Croton macrostachyus* by using different solvent like methanol, petroleum ether, ethyl acetate and chloroform. Phytochemical analysis showed the presence carbohydrate, reducing sugars, phenolics, flavonoids, glycosides, saponnins and steroids. The moisture content of the crude sample was found to be 9.01 ± 1.20 while the water extractive index was 1.14 ± 0.036**.** Quantitative analysis of phenols, flavonoids and Triterpenoids was further performed. Preliminary phytochemical screening of roots were by methanolic extracts which identified 12 major functional groups, amongst them flavonoid, phytosterols, saponins and coumarins. Additionally inorganic elements like iron, chloride and sulphate were identified by total ash analysis. The same extract was used for quantitative determination of total flavonoid content (81.88 mg/g), total phenolic content (67.78 mg/g) and total triterpenoids content (13.22mg/g). The methanolic extract had the highest antioxidant property compared to other fractions.

KEYWORDS: *Croton macrostachyus*, phytochemical screening, proximate analysis, antioxidant activity, proximate analysis, free radical scavenging activity.

INTRODUCTION

Plants are furnished with various phytochemical molecules such as terpenoids, phenolic acids, vitamins, lignins, stilbenes, tannins, amines, betalains, flavonoids, quinones, coumarins, alkaloids, and other metabolites, which are rich in antioxidant activity.[1,2] Studies have revealed that lot of these antioxidant compounds have anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial and antiviral activities.[3,4] The treatment with natural antioxidants has been connected with reduced risks of cancer, cardiovascular disease, diabetes and other diseases related to ageing[5,6] and in current years, there has been a worldwide movement towards the exercise of the natural phytochemicals present in oilseeds, beans, fruits berry crops, teas, herbs and vegetables.[7-9] In current years, phytochemicals (secondary metabolites) with unknown pharmacological activities have been comprehensively studied as a source of therapeutic agents.[10] Since there is a hurdle in use of traditional medicines worldwide due to lack of quality and quantity safety and efficacy information on traditional medicines. The lack of research data are not only due to lack of methodologies for the evaluation of herbal medicines but also due to health policies.[11] The plant contains lots of active chemical and therapeutically constituents. Hence in modern systems of medicine it important to study quality control of herbal medicines for their active chemical constituents. To satisfy new thrust of inquisitiveness, standardization of herbal medicine is compulsory.[12-16]

Now a days, there has been increasing attention in the free radical causing diseases and antioxidants. The main objective is to renew interest in the last decade to search for phytochemicals from naturalized plant for pharmaceutical and nutritional purposes. This arises from the fact that plant derived products hold great potentials as sources of pharmaceutical. Without plants, most medicines that we take would not exist. Over 40% medicine now prescribed contains chemicals derived from plants.[17] Free radicals are chemical compounds which contain an unpaired electron spinning on the peripheral layer around the nucleus. They are chemically aggressive molecules which react with different type of macro-molecules in the body to cause damage to vital

cell constituents such as DNA, proteins and lipids.[18] Free radicals react with other molecules by extracting electrons from them in order to obtain stability. Free radicals contribute more than one hundred disorders in humans including atherosclerosis and arthritis. Ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS.[19,20] The family of free radical generated from oxygen is called reactive oxygen (ROS) and those generated from nitrogen are called reactive nitrogen species (RNS). Cells are often equipped with natural mechanism to fight against ROS and to maintain redox homeostasis of cell. For example, antioxidant enzyme such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) play important roles in scavenging the free radicals and preventing cell injury.[21] The harmful effect of free radicals causing potential biological damage is termed oxidative stress. Oxidative stresses are increasingly recognized for their contribution to a number of diseases disorder such as cancer, neuron degenerative disorder, atherosclerosis and ageing among others.[22] The process of redox regulation protects living organisms from various oxidative stresses and maintains "redox homeostasis" by controlling the redox status in vivo.[23]

Antioxidants are a group of substances that when present in low concentrations compared to those of the oxidase substrate significantly delays or prevent oxidation of that substance while often being oxidized themselves.[24] Oxidation reaction can produce free radicals which in turn can start chain reactions which can cause damage or cell death. Antioxidants have the capabilities to terminate these chain reactions by removing free radical intermediates and inhabit other oxidation reaction.[25]

MATERIALS AND METHOD

Collection of plant materials

Fresh *Croton macrostachyus* roots were collected from malur.

The roots were carefully washed with water to remove earthy materials after which the bark was carefully removed, air dried and powdered using a mechanical grinder. The powder was passed through sieve No 40 and stored in air tight container for extraction.[26-28]

Extraction

Exactly 4.6 kg of the powdered sample was macerated with methanol (14 L) for 5 days. This was then filterted and evaporated in vacuo. The extract was partitioned using petroleum ether, ethyl acetate and chloroform and the different fractions stored at 4^{o}C until ready for use.

Proximate Analysis

The following quantitative parameters of *Croton macrostachyus* root sample were determined using standard methods[29,30]; Moisture content (water loss on drying), total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive value and water soluble extractive value.

Phytochemical Screening

Chemical tests to detect the presence of alkaloids, tannins, saponins, carbohydrate, protein, flavonoids and other phenolic compounds *Croton macrostachyus* roots were carried out using standard methods.[31-32]

Free Radical Scavenging Activity

The free-radical scavenging effect of crude extracts and fractions of J. multifida was estimated using DPPH scavenging method.[33] Exactly 1.0 mL of 0.1 mM DPPH was mixed with 3.0 mL of extract/fraction in methanol of concentrations 0.01-0.2 mg/mL. The reaction was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm with ascorbic acid as a reference.

Determination of total phenolic content

To determine total phenolic content from the methanolic extract of roots of *Croton macrostachyus*, calibration curve of standard Gallic acid of 20, 40, 60, 80 and 100 mg/ml was prepared in water and 1 mg/ml of methanolic extract of roots of *Croton macrostachyus* was prepared simultaneously. Each sample was mixed with 0.25 ml of Folinciocalteu reagent and 1.25 ml sodium carbonate solution. The mixtures were allowed to react for 40 minutes at room temperature. After the reaction period the blue color was measured at 725 nm on UV-visible spectrophotometer of LABINDIA 3000+ and calculated the amount of total phenolic content from calibration curve as Gallic acid.[34]

Determination of total flavonoid content

An aliquot (l ml) of standard solution of quercetin (20, 40, 60, 80 and 100 µg/ml) was added to 10 ml volumetric flask containing 4 ml of 5% $NaNO^2$ into it. After 5 minute 0.3 ml of 10% $AlCl^3$ was added. Then 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. Same dilutions were also prepared for the test solution. Blank determination was done by using methanol in place of test or standard solutions. Mixed well and taken the absorbance at 358 nm against blank. From the obtained standard curve of quercetin the total flavonoids content of methanolic extract of roots of *Croton macrostachyus* was determined.[34]

Determination of total Triterpenoids

5 g of powder extracted with 50 ml distilled water by heating on water bath for 30 min. then the extract was allow to cool and then filter. 75 ml chloroform and diethyl ether was added in 1:2 concentrations by continuous stirring for 30 min. after 5 gm of sodium carboxyl methyl cellulose was added to forms lumps and sticky mass and then separated. Further marc subjected to extraction with 75 ml chloroform: diethyl ether (1:2) for four times. The obtained residue was dissolved in 50

ml of neutral absolute alcohol. Then the mixture were titrated with 0.1 N NaOH using phenolphthalein as an indicator. Similarly blank readings were taken without addition of sample. Percentage of Triterpenoids content was calculated as per the given factor. Factor for the calculation: each ml of 0.1N NaOH = 48.8 mg of Triterpenoids.[34]

RESULTS

Phytochemical analysis		
Sl.No	**Test**	**Inference**
1	Carbohydrate	+
2	Protein	+
3	Glycoside	-
4	Saponin	+
5	Coumarin	+
6	Flavonoid	+
7	Anthraquinone glycoside	-
8	Phytosterol	-
9	Phenol	+
10	Alkaloids	-
11	Lipid	+
12	Steroids	+

+ indicates presence of compound
– indicates absence of compound

Table 01: Results of phytochemical analysis of *Croton macrostachyus* root

Phytochemical analysis		
Sl.No	**Test**	**Inference**
1	Calcium	+
2	Iron	+
3	Magnesium	-
4	Potassium	+
5	Sulphate	+
6	Phosphate	+
7	Chloride	-
8	Carbonate	+
9	Nitrate	+

(+) present (-) absent

Table 02: Detection of inorganic elements.

Parameter	Value± SEM (%)
Moisture Content	9.01 ± 1.20
Water extractive index	1.14 ± 0.0367
Total Ash	9.99 ± 0.0290
Acid Insoluble Ash	8.83 ± 0.0199
Alcohol extractive index	0.49 ± 0.0280

Table 3: Percentage (%) values of proximate analysis of *Crotonmacrostachyus* root sample

Sample	IC50 value (µg/mL)
Methanol extract	249.98
Pet – Ether fraction	178.21
Ethyl acetate fraction	88.79
Chloroform fraction	35.45
Ascorbic acid	4.01

Table 4: The IC-50 values of the different fractions of the root extract of *Croton macrostachyus*

***Croton macrostachyus* (Roots)**	
Total flavonoids	81.88*
Total phenols	67.78*
Total Triterpenoids	13.22*

Table 05: Quantitative analysis of phytochemicals (mg/g)

DISCUSSION

The phytochemical screening of the powdered root of *Croton macrostachyus* revealed the presence of carbohydrate, protein, saponin, coumarin, flavonoid, phenol, lipid and Steroids (table 1) and inorganic elements like calcium, iron, potassium, sulphate, phosphate, carbonate and nitrate (table 2). Hence roots of plants can also be used as source of medicine for treatment of many diseases.

In this study, proximate analysis was carried out for the purpose of authentication of the crude powdered plant material. The maximum permissible range of moisture content for a crude drug is between 6 – 8% (African Pharmacopoeia, 1986). The total ash is a measure of the non-volatile inorganic constituents remaining after ashing. A moisture content of 9.01 ± 1.20% (table 3) obtained from this study suggest that the crude plant material is not susceptible to microbial degradation or hydrolytic break down of the chemical constituents.

The DDPH Assay is based on the measurement of the reducing ability of antioxidant towards DPPH. This ability can be evaluated by electron spin resonance (ESR) spectrometry or by measuring the decrease of the absorbance.[35] The DPPH assay is considered to be mainly based on electron transfer (ET) reaction and hydrogen atom abstraction is a marginal reaction pathway.[36]

The result of the DPPH showed that extracts have appreciable DPPH radical scavenging activity with the chloroform fraction having the lowest scavenging. The 50% inhibitory concentration (IC50) of the Chloroform extract was significantly lower than values obtained from the other fractions and the crude extract (table 4). The scavening activity also increased as the concentration of the extract increased.

The antioxidant activity could be attributed to presence of phenolic compound present.

CONCLUSION

From the study, the root of *Croton macrostachyus* possess significant antioxidant property and could be a potential source of antioxidant drug.

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