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RESEARCH ARTICLE

ASSESSMENT OF TOTAL PHENOL AND FLAVONOID CONTENT OF COSTUS SPECIOSUS AND CATHARANTHUS ROSEA IN DIFFERENT GEOGRAPHICAL REGIONS OF BALAGHAT DISTRICT, MADHYA PRADESH

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Abstract

Phenols and flavonoids are secondary metabolites of the plant and play a key role in scavenging free radicals and act as antioxidant. Costus speciosus (Costaceae family) and Catharanthus rosea (Apocynaceae family) are important ornamental and ethno medicinal plant rich in bioactive compounds used for treatment of several types of diseases. The plants are well known for their various pharmacological activities such as anti-diabetic, anticancer, antibacterial, antifungal, antioxidant, anti-hyperglycemic, anti-inflammatory, analgesic, antipyretic, anti-diuretic, antiulcer and anti-diarrheal activities. In the present study the total phenol and flavonoid content of root methanol extract of both plants were determined from five blocks of Balaghat district Madhya Pradesh. Folin-Ciocalteu assay was used to determine total phenol content and aluminium chloride method for flavonoid content using gallic acid and quercetin as standard respectively. The result showed highest concentration of total phenol content in Birs block for both C. speciosus (95.85±1.56 mg GAE/g) and C. rosea (82.32±0.81 mg GAE/g). The total flavonoid content was found highest in Parawada block for C. speciosus (97.67±0.53mg/QE) and Baihar block for C. rosea (93.20±0.54mg/QE). The ratio of flavonoid to the phenolic in each extract was also determined to as certain extracts that are rich in flavonoids.

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Introduction:-

Costus speciosus (Koen.) is an important medicinal and ornamental plant grown in sub Himalayan region of central India and part of Western Ghats of Maharastra, Karnataka and Kerala. It belongs to the family Costaceae which are the largest genus of Costus and originated from Malaya peninsula of South Asia. The plant is popularly known as “crepe ginger”. It can grow up to 5 feet tall in frost free areas but cooler region grow up to 6 feet tall. The genus Costus includes 175 species found in all over the world but approximately 100 species are found in India, out of them only seven C. barbatus, C. chartaceus, C. cuspidatus, C. giganteus, C. igneus, C. osae and C. spectabilis are under the cultivation in India. Costus species are generally grown in productive, organic, moist, well-drained soils in shade (Whistler, 2000). C. speciosus contains several phytochemicals such as steroids, phenolic, alkaloids, glycosides (Singh et al., 2014), polyphenols, tannins, flavonoids, and β-carotene (Pai Kotabagilu et al., 2014),

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furostanol, saponins-costusosides, dioscin, gracillin, dihydrophytylplastoquinone, diosgenin, β -sitosterol, β -D-glucoside, prosapogenins, and α -tocopherolquinone (Lijuan et al., 2011). It has been reported that the plant contain β -amyrin, camphene, costunolide, diosgenin, α -Humulene, lupeol, and zerumbone having anticancer activity (Saleem, 2009; Zhang et al., 2012). The rhizome also contains secondary metabolites like phenol, flavonoids, tannin, saponin, steroids, and glycoside which are studied for a number of biological consequences including antimicrobial and antioxidant activities (Borkataky et al., 2014). The rhizomes of *Costus speciosus* are bitter in taste and exhibit various activities such as antihelmintic, astringent, expectorant and used mainly for treating diabetes. It has been reported that rhizome extract was used for treating burning sensation, constipation, leprosy, asthma, bronchitis, anaemia and other skin ailments (Bown Deni, 2008). Rhizomes of plant were used as herbal remedy for fever and its paste were used for treating boils. Leaves of the plant were used for scabies and stomach ailments. Besides rhizomes, stems are also used for treating blisters and burns and roots are used against snake bite (Gruenwald et al., 2000). *Catharanthus roseus* belongs to family Apocynaceae and is a dicotyledonous angiosperm plant which synthesizes two terpene indole alkaloids namely vincristine and vinblastine that act against cancer (Ajaib, 2010). More than 100 monoterpenoidindole alkaloids (TIA) are produced by these plants in different organs. *Catharanthus roseus* have 400 alkaloids, and alkaloids are the most active components of the plant used in pharmacological activities, flavor and fragrance, ingredients, food additives, pesticides and agrochemicals (Bernett et al., 1978). The ariel parts of the plant contain alkaloids like vindesine, vindeline tabersonine, vinblastine, vincristine, whereas raubasine, reserpine, catharanthine, vinceine, vineamine, and ajmalicine are present in basal or root parts of the plant. About 150 alkaloids of pharmacological importance are present on plant leaves. In laboratory animals significant antihyperglycemic and hypotensive activity was reported by the leaf extracts of *Catharanthus roseus*. The plant has good antioxidant potential and contain significant amount of volatile and phenolic compounds such as caffeoylquinic acids and flavonal glycosides which act as antioxidants against reactive oxygen species. These compounds also exhibit anti-inflammatory, antimicrobial, antioxidant, antithrombotic, anti-allergic, vasodilatory effects and cardio-protective properties (Kumar et al., 2015). In 2007 Mustafa and Verpoorte reported a number of phenolic compound such as 2, 3-dihydroxybenzoic acid, and phenylpropanoids such as flavonoids, anthocyanins and cinnamic acid derivatives in *Catharanthus roseus*. Phenol and flavonoid compounds are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipooxygenase and scavenge free radicals. Currently, plant materials rich in antioxidants are used in the food industry because they decrease the oxidative degradation of lipids and maintain the quality and nutritional value of food (Kahkonen et al. 1999). The aim of the present study was to determine the total phenol and flavonoid content in methanol root extracts of *Costus speciosus* and *Catharanthus rosea* collected from five blocks of Balaghat district, Madhya Pradesh.

Materials and Methods:-

Plant collection:

The plant samples of *Costus speciosus* and *Catharanthus rosea* were collected from natural environment from five block of Balaghat district, Madhya Pradesh (Table1) and authenticated by Botanical Survey of India, Allahabad.

Preparation of extracts:

The root part of the plants was washed, dried under shade and grinded to make powder. The methanol extract of both plants were prepared using Soxhlet apparatus (MSW, India) according to method developed by Wagner et al. (1996). After extraction each extract was stored in air tight bottles and kept in refrigerators for further studies.

Table1:- Localities and their attributes selected for collection and study of *Costus speciosus* and *Catharanthus rosea*.

S.No.	Sample collection sites	Average temp(°C)	Lattitude	Longitude
1.	Birsa (Kaniya)	28 °C	21°50' N	080°50' E
2.	Baihar (Pathri)	30 °C	21°20' N	080°42' E
3.	Parswada(Geegali ghoghara)	29 °C	22°09' N	080°14' E
4.	Balaghat (Gangulpara)	34 °C	21°53' N	080°18' E
5.	Lanji (Devarbeli)	31 °C	21°37' N	080°39' E

Determination of total phenolic content (TPC):

The total phenol content in plant extracts were determined by Folin-Ciocalteu assay method using gallic acid as standard with slight modification (Singleton et al., 1999). The reaction mixture consists of 1 ml of extract and 9 ml of distilled water was taken in a volumetric flask (25 ml) and then 1ml of Folin-Ciocalteu phenol reagent was mixed with the mixture and shaken well. After 5 minutes, 10 ml of 7 % sodium carbonate (Na_2CO_3) solution was treated to the mixture. The volume was made up to 25 ml. A set of standard solutions of gallic acid (20, 40, 60, 80 and 100 $\mu\text{g/ml}$) were prepared. Incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 650 nm with an ultraviolet (uv) /visible spectrophotometer. The total phenol content was determined from calibration curve (Fig1) and expressed as mg of GAE/gm of extract.

Determination of Total flavonoid content (TFC):

Aluminium-chloride spectrophotometric method was used to determine total flavonoid content. The plant extract of 0.25mL was dissolved in 1.25mL distilled water then NaNO_3 was added. This mixture was placed in dark for 6 minutes then 0.150 ml of 10% AlCl_3 was added in the mixture and again incubated in dark for 5 minutes. Now 0.5 ml NaOH and 0.275 ml distilled water was added in the mixture. The set of standard solutions of quercetin (20, 40, 60, 80 and 100 mg/ml) were prepared in the same manner as described for the extracts. The absorbance of standard and the extracts solutions were measured against the reagent blank at 510 nm with a UV/Visible spectrophotometer. The total flavonoid content was determined from the calibration curve (Fig2) and expressed as milligram of quercetin equivalent (QE) per gram of extracts.

Statistical Analysis:

The experiments were carried out in three replicates and data were presented as mean \pm S.E. in Microsoft Excel 2010 for statistical and graphical evaluations.

Results and Discussion:-**Total phenolic contents:**

The Folin-Ciocalteu reagent was used to determine total phenol contents which reduce polyphenols. Phenols present in plants react with phosphomolybdic acid in Folin-Ciocalteu reagent in alkaline medium and produce a blue coloured complex (Molybdenum blue). Spectrophotometer was used for quantification of this blue colour complex and thus total phenolic content was determined (Kale et al., 2010). In the present work methanol root extract was used for determination of total phenol content in both plants. The regression equation for the calibration curve of standard gallic acid was ($y = 0.005x - 0.002$, $R^2 = 0.999$) as shown in (Fig1). The total phenol content of *C. speciosus* was found highest in plant collected from Birsa block with a concentration of (95.85 ± 1.56) mg/GAE/g extract and least in Lanji block (59.59 ± 1.23) mg/GAE/g extract. In *C. rosea* highest concentration of total phenol content was found in Birsa block (82.32 ± 0.81) mg/GAE/g and least in Lanji block (71.03 ± 1.97) mg/GAE/g extract. Nehete et al. in 2010 studied the antioxidant activity of *C. speciosus* and found significant phenolic content and antioxidant activity in benzene extracts of rhizome because it contains a maximum phenolic content of 4.38%. Similarly in our study we have reported the significant concentration of total phenol in root extracts of *C. speciosus* (95.85 ± 1.56) mg/GAE/g and *C. rosea* (82.32 ± 0.81) mg/GAE/g which suggests its antioxidant activity. Rasool et al in 2011 reported that highest antioxidant activity was exhibited by ethyl extract of *C. rosea*.

Total Flavonoid content:

Flavonoids as one of the most diverse and widespread group of natural compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. The total flavonoid content of plant was determined by aluminium chloride method (Table 2 and 3). The aluminium chloride form stable complexes with C-4 keto group or either the C-3 or C-5 hydroxyl groups of flavonoids in presence of NaNO_3 in alkaline medium which has absorption maximum at 510nm (Kale, 2010). The regression equation for the calibration curve of total flavonoid was ($y = 0.007x - 0.003$, $R^2 = 0.999$) as shown in Fig2. The highest concentration of total flavonoid in *C. speciosus* was found in plant collected from Paraswada block (97.67 ± 0.53) mg QE/g whereas lowest from Lanji (80.82 ± 0.29) mg QE/g. In *C. rosea* highest concentration was found in Baihar block (93.20 ± 0.54) mg QE/g and lowest in Lanji (70.71 ± 0.28) mg QE/g.

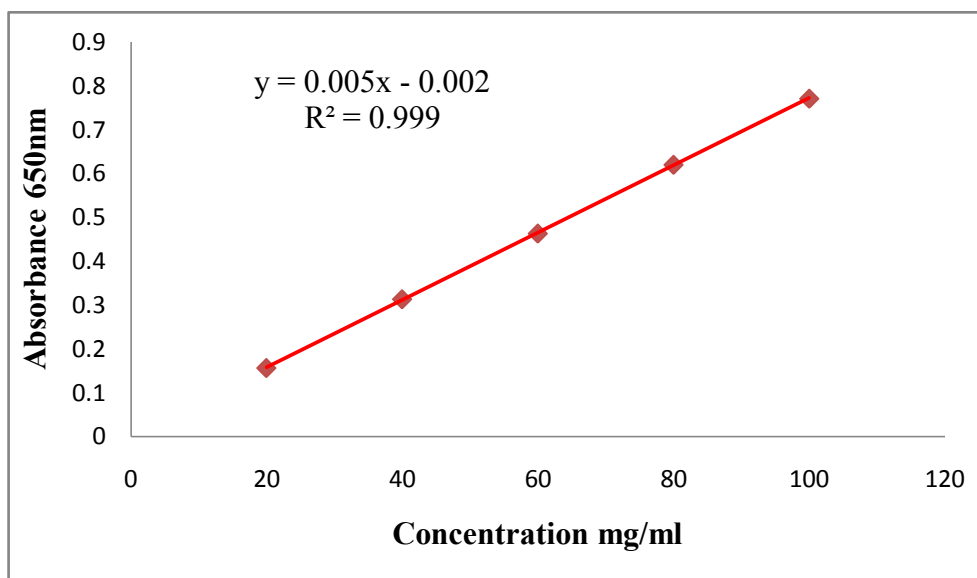


Fig 1:- Calibration curve for phenol standard (gallic acid).

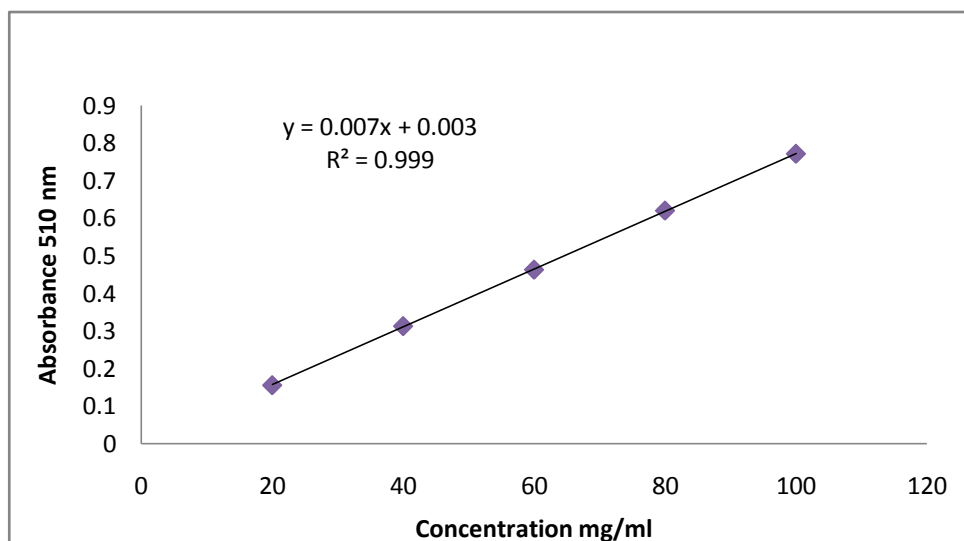


Fig 2:- Calibration curve for flavonoid standard (quercetin).

Table 2:- Total phenol and flavonoid contents in methanol root extracts of *C. speciosus*.

S.	Sample	Total phenols (mg GAE/g of extract)	Total flavonoids (mg QE/g)	Flavonoid/ Phenol (F/P) ratio
1.	Birsa	95.85±1.56	91.21±0.41	0.951
2.	Baihar	80.92±1.45	94.30±0.43	1.165
3.	Paraswada	76.56±1.88	97.67±0.53	1.275
4.	Balaghat	63.22±1.47	82.90±0.35	1.311
5.	Lanji	59.59±1.23	80.82±0.29	1.356

Table 3:- Total phenol and flavonoid contents in methanol root extracts of *C. rosea*.

S.	Sample	Total phenols (mg GAE/g of extract)	Total flavonoids (mg QE/g)	Flavonoid/ (F/P)ratio	Phenol
1.	Birsa	82.32±0.81	82.85±0.39	1.006	
2.	Baihar	77.60±1.60	93.20±0.54	1.201	

3.	Paraswada	75.36±1.18	71.86±0.16	0.953
4.	Balaghat	73.92±1.15	75.97±0.68	1.027
5.	Lanji	71.03±1.97	70.71±0.28	0.995

Each value is the average of three analyses ± standard deviation.

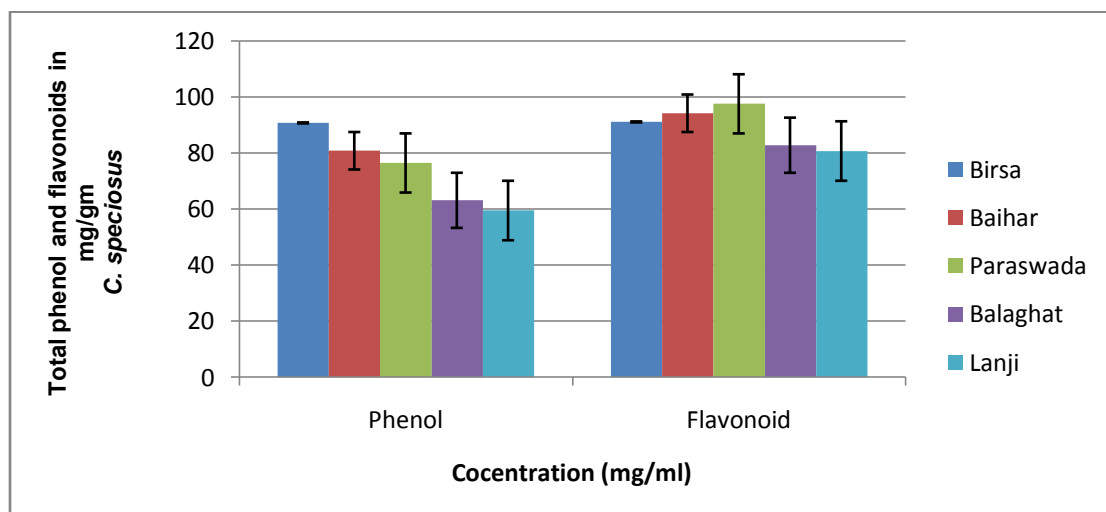


Fig 3:- Showing total phenol and flavonoid content in mg/ml of *C. speciosus* from five blocks of Balaghat district.

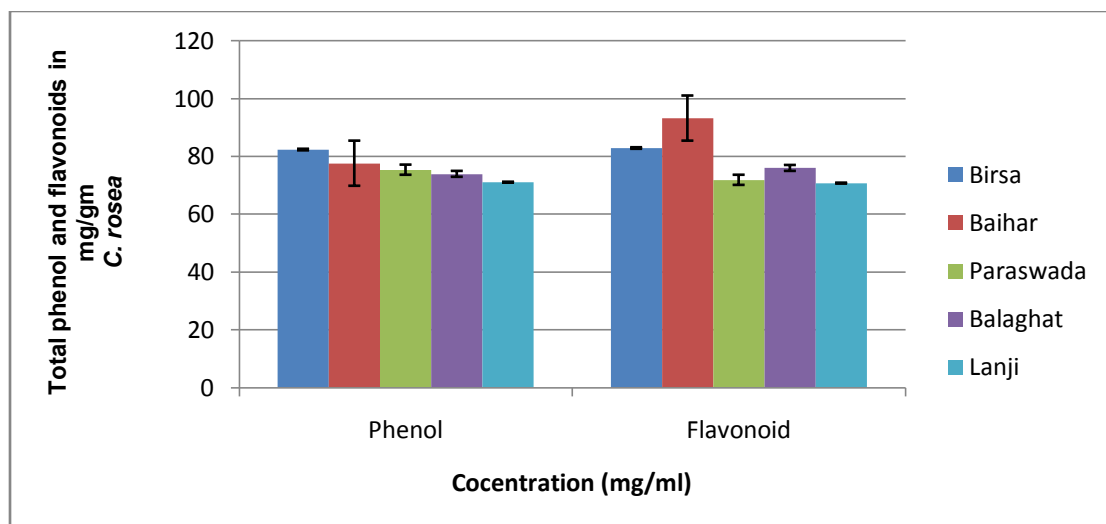


Fig 4. Showing total phenol and flavonoid content in mg/ml of *C. rosea* from five blocks of Balaghat district.

The total flavonoid to phenolic ratio in each extract was also determined to ascertain extracts that are rich in flavonoids which were found highest in Lanji block (1.356) in *C. speciosus* and Baihar block (1.201) in *C. rosea*. The concentration of phenol and flavonoid constituents of the plants varies in different regions of Balaghat district which might be due to the influence of environmental conditions. The presence of polyphenols in plants is essential because they are natural antioxidants (Zheng and Wang, 2001). In 2013 Kefeli et al. reported that presence of phenols in plants exhibit antibacterial properties. Several authors have previously reported the curative properties of plant phenols in treatment of various ailments. Flavonoids have potent free radical scavenging activity which prevent oxidative cell damage and showed anticancer activity (Doss, 2009). The antioxidant activity of the plant was due to the presence of phenolics and flavonoid which are known for their free radical scavenging activity. Flavonoids exhibits wide range of biological activities such as antimicrobial, anti-inflammatory, anti allergic, anti analgesic cytostatic, and anti oxidant properties. Our present investigation showed high phenolic and flavonoid content in root extract of *C. speciosus* and *C. rosea* which may suggests significant antioxidant and antimicrobial activities of both plants.

Conclusion:-

Costus speciosus and *Catharanthus rosea* are multipurpose medicinal plant used in traditional and modern medicines for treatment of many health problems. The present work explained the total phenol and flavonoid content of root methanol extracts of both plants in different location of Balaghat district Madhya Pradesh. The concentration of phytochemicals varies greatly due to environmental effects. A significant concentration of phenol and flavonoid content was found in both plants which showed its correlation with antioxidant potential. This study should be beneficial for characterization and structure elucidation of pharmaceutically lead compound.

Conflict of interest:

The authors declare no conflict of interests.

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