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Invitro Quantification of Flavonoids and Phenolic content of – Suran

H.N.Nataraj*[1], R.L.N.Murthy[1] and Dr.S. Ramachandra Setty[2]

[1]T.V.M. College of Pharmacy, Bellary – 583 103,India.

[2]S.C.S. College of Pharmacy, Harapanahalli – 583 131,India.

Corresponding Author: hn.nataraj@yahoo.com
Mobile no.:09886798060

Abstract: Suran-Tuberous one being used in the treatment of various human ailments including liver cirrhosis, cataract, haemophillic conditions, debility, in vitiated conditions of Vata, Kapha etc., in Ayurvedic system of Medicine. Keeping this information in view, the Methanolic extract (ME) and 70%HydroAlcoholic extract (AE) of Suran - tubers of "Amorphophallus paeoniifolius" (Donnst) Nicolson family (Araceae) was analysed for Flavonoidal content (FC) in terms of Rutin and Total phenolic content (TPC) was measured in terms of Catechol equivalent. TLC study of Methanolic extract was conducted. The Flavonoidal content of ME & AE were found to be 46.33 mg/g and 36.88 mg/g respectively. Similarly TPC of study extracts (ME & AE) were found to be 12.67 mg/g and 6.25mg/g. However the flavonoidal and phenolic contents of ME was found to be higher. Upon TLC of the ME it was observed that there were seven spots at different R_f values. Further studies are in progress to ascertain that the phenolic content of the extract contribute for the therapeutic properties of the 'Suran'.

Keywords: Total flavonoids, Total phenolic content, Amorphophallus paeoniifolius.

Introduction

Recently much attention has been focused on reactive oxygen species and free radicals which play an important role in the genesis of various diseases such as inflammation, cataract, liver cirrhosis, ischemia/reperfusion injury, cancer, etc.[1] Flavonoids and Phenolics are the bioactive phytoconstituents having an important role in control & prevention of tissue damage by activated oxygen species.[2] And hence herbal drugs / herbal preparations containing such phytoconstituents are gaining importance in the prevention and treatment of various organ toxicities due to xenobiotic / environmental challenges.[3]

Amorphophallus paeoniifolius (Dennst) Nicolson (Araceae) a tuberous, stout indigenous herb commonly krown as elephant foot yam, Suran, grown as vegetable is widely available[4-5] and is reported to contain flavonoids.[6] In Ayurvedic System of Medicine tubers of this plant has been indicated in treating various above mentioned pathophysiological conditions due to ROS[7-12]. Tubers are reported in management of haemmrroids[13] to have antiprotease activity[14], antiomicrobial activity[15] and analgesic activity of its methanolic extract[6].

This exhaustive literature survey revealed that the tubers are not yet screened for its quantitative evaluation of Flavonoids & Total phenolic contents of the extracts of the tubers. Hence in the present study an attempt is made to standardize the plant material in terms of its Flavonoidal content and Total phenolic content.

Material and Methods

Collection of Plant material and preparation of extracts

The tubers of Amorphophallas paeoniifolious were collected from cultivated lands from Hassan district of Karnataka and authenticated by Dr. Kotresh, Botany department, Karnataka University constituent college, Dharwad. The voucher specimens of these plants and tubers were preserved in the herbarium of the pharmacognosy department of this institution.

The air dried powder of tubers was subjected to exhaustive soxhlation with solvents Methanol and 70% Hydroalcohol separately. Later solvent was evaporated on rotary vaccum evaporator below 50^0C temperature to get reddish brown methanol extract (ME) and dark brownish 70% hydroalcoholic extract (AE).

Preliminary phytochemical screening

Both the extracts (ME & AE) were screened for the presence of various secondary metabolites mainly tannins, flavonoids, coumarins, polyphenols, sterols and triterpenoids using standard qualitative tests.[16-17] These extracts were subjected for quantification of their FC and TPC.

Experimental

Total Flavonoidal content (FC)

To determine the total flavonoidal content, stock solutions of the both extracts (ME: 77mg/ml; AE: 44mg/ml) were prepared with methanol to a suitable concentration for analysis. Total flavonoid content was measured according to the method previously reported by Helmija et al.,[18] with slightly modifications using standard curve generated with Rutin.

Aliquots of each extract (ME & AE) were pipetted out in series of test tubes and volume was made upto 0.5ml with distilled water; Sodium nitrate (5% : 0.3ml) was added to each tube & incubated for 5 min. at room temperature; Aluminium chloride solution (10%; 0.06ml) was added and incubated for 5 min, at room temperature; Sodium hydroxide (1M; 0.25ml) was added and total volume was made to 1ml with distilled water. Absorbance was measured at 510nm against a reagent blank using Schimadza model 150 – 02 double beam spectrophotometer and concentration of flavonoids in the test sample was determined and expressed as mg of Rutin equivalent per gram of sample.

Total Phenolic Content (TPC)

Total phenolic content was assessed approximately by using Folin-Ciocalteau Phenol reagent according to the method previously reported by Malik and et al.,[19] using standard curve generated with Catechol.

To determine the total phenolic content, the stock solutions of both extracts (ME: 45mg/ml; AE: 40mg/ml) were prepared in methanol to a suitable concentration for analysis. Aliquots of each sample were pipetted out in series of test tube and volume was made upto 3 ml with distilled water. Folin ciocalteau reagent (0.5ml) was added to each tube and incubated for 30 mins. at room temperature; Sodium carbonate (250% w/v, 2 ml) was added, mixed thoroughly and the tubes were incubated for 1min. in boiling water bath. Absorbance was measured at 650nm against a reagent blank. TPC was expressed as mg of Catechol equivalent per gram of sample.

TLC study of methanolic extract

The methanolic extract was analysed for flavonoids and coumarins by running Thin Layer Chromotography of the extract on Silica gel 60F 254 precoated sheets with two different solvent systems separately[20].

a) The methonolic extract was chromotographed with BAW (n-butanol:Acetic acid:water ; 4:1:5, top layer) as mobile phase. The developed plates were dried, sprayed with NP/PEG and observed under uv light at 365nm. Later plates were sprayed with acid (10% H_2SO_4 in methanol), heated at 110^0C and again observed.
b) Similarly the extract was subjected to chrmotography with 10% OHAc (Acid acid) as mobile phase and treated as erlier. (Fig : 2,b)

The R_f values of resolved spots were documented in Table 4.

Results and Discussion

Plants are conceived as sources of antioxidants due to presence of polyphenols and flavonoids which possess wide biological properties.[21] Recent studies showed that many flavonoids & related polyphenols contribute significantly to the total antioxidant activity of many plants.[22]

The preliminary phytochemical screening of the both (ME & AE) extracts shown that they contain flavonoids, coumarins, sterols, tannins and triterpenoids. However it was observed that the Total Phenol Content (TPC) was found to be very much higher than the non polar constituents like sterols. The results of preliminary phytochemical screening is compiled in Table No.1.

Flavonoids content was found to be 46.33 mg/g and 36.88 mg/g in terms of Rutin equivalent for ME & AE respectively. The standard curve of Rutin is given in Fig.3 and total flavonoidal content is documented in Table No.2.

Similary the TPC of ME was found to be 12.67 mg/g and of AE 6.25 mg/g interms of Catechol equivalent. The standard curve of Catechol was given is Fig.4 and TPC is discumented in Table No.3.

The TLC of ME revealed the presence of seven spots at different R_f values with n-Butanol: Acetic Acid: Water (4:1:5) solvent system. Further only three spots were seen with 10% Acetic Acid solvent system indicating that the extracts contain three coumarins and remaining were flavonoids. These reports are indicating that total phenolic content is directly proportional to antioxidant activity of the tuberous plant. The flavonoids and coumarins are also contribute to the TPC.

Conclusion

In the present study the total phenol content & total flavonoidal content were determined and this in terms helps in gauging the antioxidant potential of the tuberous plant. In addition to this the present findings are not only helpful for establishing the phytochemical standardization but also in authentication of this drug.

Table No. 1: Details of Qualitative Phytochemical Tests

Tests for Phytocontituents	Methanol extract	70% Hydroalcol extract.
Tests for Tannins & Polyphenolics		
1) Ferric chloride test	+ +	+ +
2) Gelatin Test	+ +	+ +
3) Lead Acetale Test	+ +	+ +
4) Bromine water	+ +	+
5) Dil. Pot. Permanganate	+ +	+
6) Dil. Iodine	+ +	+
7) Dil. HNo_3	+ +	+ +
Tests for Flavonoids		
1) Ferric Chloride test	+ + +	+ +
2) Shinoda Test	+ + +	+ +
3) Lead Acetate test	+ + +	+ +
4) Alkaline Reagent Test	+ + +	+ +
Tests for coumarins		
Fluorescence test	+ +	+
Tests for sterols		
1) Salkouski's Test	+	+
2) Libermann Buchard's Test	+	+
3) Liberman's Test	+	+
4) Sulphur Test	+	+
Tests for triterpenoids	+	+

Note: '+ + +' indicates highly significant, '+ +' indicates significant, '+'indicates presence.

Table 2: Flavonoidal content of extracts

Extracts	Absorbance at 510nm	Rutin equivalent flavonoids (decived from graph)	Rutin equivant flavonoids (mg/ml) present in stock	mg of Rutin equivalent (calculated from mg/ml of stock)
AE	0.66 (25 µl of stock)	71 µg / 25µl	2.84	36.88 mg/g
ME	0.48 (25 µl of stock)	51 µg / 25µl	2.04	46.33 mg/g

Table 3: Total Phenolic content of extracts.

Extracts	Absorbance at 650nm	mg of Catechol equivalent
AE	0.13	6.25 mg/g
ME	0.29	12.67 mg/g

Table 4: TLC screening of Methonlolic extract of tuber of Amorphophallus paeoniifolius.

Adosorbent	Solvent system	Detecting Reagent	Observation	Inference	Rf Values	
					Under UV light 365nm	After acid spray and heated at 110^0C
Silica gel 60GF 254 precoated sheet	n-Butanol: Acetic Acid : Water (4:1:5)	NP/PEG & UV	Yellow/ Orange	Flavonoids present	0.31, 0.27,0.41, 0.65,82, 0.92	0.27,0.31, 0.41,0.51, 0.65,0.82, 0.92
		NH_3/KOH	Light Blue	Coumarins present		
Silica gel 60GF 254 precoated sheet	10% Acetic Acid	NP/PEG & UV	----	Flavonoids absent	0.27	0.13, 0.27, 0.92
		NH_3/KOH	Deep Blue	Coumarins present		

Fig 1: Photograph of Suran.

Fig 2,b. TLC Chromotogram of MeOH extract under uv 365nm

Mobile phase (10% OHAc)

Fig 3 : Rutin Calibration Curve

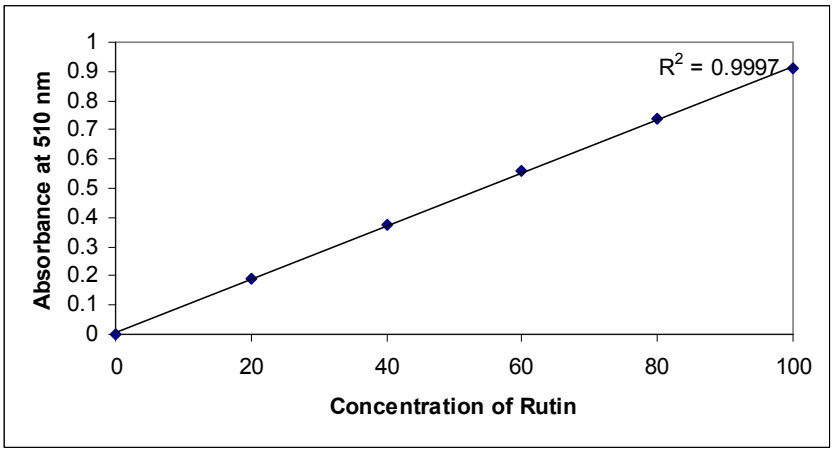
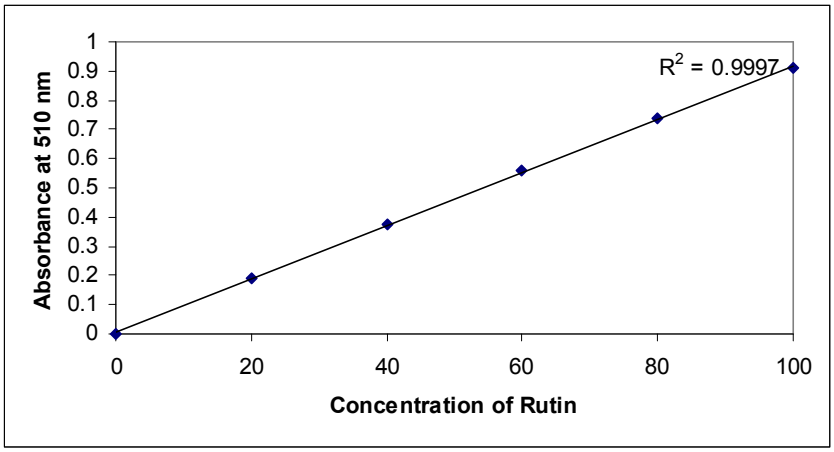


Fig 4 : Catechol calibration curve



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