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Investigation of phytochemical and antioxidant property of Stereospermum colais

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Abstract: **Stereospermum colais leaves were extracted with hexane, acetone and aqueous. These extracts were subjected to antioxidant activity by DPPH scavenging method. The rate of oxygen scavenging and phytochemical properties was identified with the same extracts. The aqueous showed higher rate of oxygen scavenging than acetone and hexane. The leaves are found to be having the wound healing properties.**

Keywords: **Stereospermum colais, Antioxidant – DPPH assay, Phytochemical, BHT.**

I. INTRODUCTION

Plants have evolved the ability to synthesize chemical compounds that help them defend against attack from a wide variety of predators such as insects, fungi and herbivorous mammals. *Stereospermum colais* is used most commonly as an anti-inflammatory and antimicrobial agent. The juice of the leaves is used in diseases of ear, teeth and in rheumatism. An attempt has been made to evaluate the antioxidant potential of the leaves of *S.colais* by DPPH scavenging method. The preliminary phytochemical screening of various extracts of the leaves shows the presence of terpenes, steroids, flavonoids, glycosides, carbohydrates, tannins and phenol. An antioxidant is a molecule capable of inhibiting the oxidation of other molecule. The total phenol flavonoid and tannin content of the aqueous, acetone and hexane extracts of *Stereospermum colais* leaf is identified.

II. MATERIALS AND METHODS

Phytochemical analysis:

Sample was added to each solvent and incubated for overnight. Then the extracts were filtered and filtrates were dried under shade. The concentrated extract were weighed and dissolved in respective solvents. These extracts were subjected to following phytochemical test such as carbohydrates, tannins, saponins, flavonoids, alkaloids, anthocyanin and betacyanin, quinines, glycosides, cardiac glycosides, terpenoids, triterpenoids, phenols, coumarins, acids, protein and aminoacids, steroids and phytosteroids, phlobatannins, anthraquinones.

Antioxidant activity:

DPPH(1, 1-Diphenyl-2, Picryl-Hydrazyl) was purchased from sigma, USA. Antioxidant activity or free radical scavenging activity of the methanolic extract against DPPH was measured according to George et al. (1996). The percentage inhibition of DPPH radical by the sample was calculated using the following formula

$$\text{Inhibition\%} = \frac{[\text{absorbance of control (A517)} - \text{absorbance of sample (A517)}]*100}{\text{Absorbance control(A517)}}$$

If free radicals have been scavenged DPPH will degenerate to yellow colour. The extracts were dissolved in methanol in the concentration of 1mg/ml and was then used to determine its antioxidant activity.

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BLANK : Methanol

CONTROL : Methanol + DPPH

TEST : Methanol + DPPH + sample

STANDARD : Methanol + DPPH (0.1%) + BHT (0.16%)

Determination of total phenolic content:

The amount of phenolic compounds in the extracts was determined by Folinciocalteu colorimetric method and calculated from a calibration curve obtained with Gallic acid as standard (10mg/10ml). The absorbance was measured at 765nm

Determination of total tannin:

Estimation of tannin concentration in the extract was measured by Folin-Denis method (Schanderi 1970) with minor modifications. The absorbance was measured at 700nm and the concentration of tannin in the extract was determined using pure tannic acid as standard.

Determination of total flavonoid:

Aluminium chloride colorimetric method was used for flavonoids determination (chang et al., 2002). The absorbance was measured at 415nm. The content of flavonoid was expressed in mg/g (quercitin(10mg/100ml) – standard

III. RESULTS AND DISCUSSION

Phytochemical analysis:

The results of the phytochemical test are presented in table1 and the inferences in table 2.

Table 1. Results of phytochemical test

Phytochemicaltest	Observation		
	Hexane	Acetone	Aqueous
Carbohydrate	Red colour	Red colour	Red colour
Tannins	Greenish black colour	Greenish black colour	Greenish black colour
Saponin	No colour change	Presence of foam	Presence of foam
Flavonoid	Yellow colour	Yellow colour	Yellow colour
Alkaloid	Green colour	Green colour	Green colour
Anthocyanin And Betacyanin	Yellow colour	Yellow colour	Yellow colour
Quinones	Red colour	Red colour	Red colour
Glycosides	No colour change	No colour change	No colour change
Cardiac Glycosides	No colour change	Brown ring	No colour change
Terpenoids	No colour change	Red brown colour	No colour change
Triterpenoids	Blue green colour	Blue green colour	No colour change
Phenols	No colour change	Green colour	No colour change
Coumarins	Yellow colour	Yellow colour	Yellow colour
Acids	No colour change	No colour change	No colour change
Proteins	No colour change	No colour change	No colour change
Steroids And Phytosteroids	No colour change	Brown ring	No colour change
Phlobatannins	No colour change	No colour change	No colour change
Anthraquinones	No colour change	No colour change	No colour change

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Table 2. Inference of phytochemical test

Phytochemicaltest	Inference		
	Hexane	**Acetone**	**Aqueous**
Carbohydrate	Strongly present	Strongly present	Strongly present
Tannins	Slightly present	Slightly present	Slightly present
Saponin	Present	present	present
Flavonoid	Strongly present	Strongly present	Strongly present
Alkaloid	Strongly present	Strongly present	Slightly present
Anthocyanin And Betacyanin	Presence of anthocyanins	Presence of anthocyanins	Presence of βcyanins
Quinones	Strongly present	Strongly present	Strongly present
Glycosides	Absent	Absent	Absent
Cardiac Glycosides	Absent	Strongly present	Absent
Terpenoids	Absent	Strongly present	Absent
Triterpenoids	Strongly present	Strongly present	Absent
Phenols	Absent	present	Absent
Coumarins	Strongly present	present	Strongly present
Acids	Absent	Absent	Absent
Proteins	Absent	Absent	Absent
Steroids And Phytosteroids	Absent	Presence of steroids	Absent
Phlobatannins	Absent	Absent	Absent
Anthraquinones	Absent	Absent	Absent

Determination of total phenolic content:

The results of estimation of total phenols against a standard Gallic acid is presented in table 3.

Table 3. Estimation of gallic acid(standard)

S.No	Absorbance at 765nm	Gallic acid concentration (mg/ml)
1.	0.168	0.02
2.	0.46	0.05
3.	0.614	0.1
4.	0.955	0.15
5.	1.248	0.2

Estimation of sample:

S.No	Absorbance at 765nm	Concentration of the sample (mg/ml)	Total phenol in GAE (mg/g)
1.	1.566	0.1	0.500

Determination of total tannin:

The results of estimation of total tannin against a standard is presented in table 4.

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Table 4. Estimation of tannin

S.No	Absorbance at 700nm	Concentration of standard (µg)
1.	0.12	20
2.	0.43	40
3.	0.63	60
4.	0.81	80
5.	1.22	100
6.	1.46	120

Estimation of sample:

S.No	Solvent	Absorbance at 700nm	Total tannin in TAE(µg/g)
1.	Aqueous	1.403	20

Determination of total flavonoid:

The results of estimation of total flavonoids against a standard is presented in table 5.

Table 5. Estimation of flavonoids

S.No	Absorbance at 415nm	Concentration of standard (µg)
1.	0.5	20
2.	0.9	40
3.	1.3	60
4.	1.7	80
5.	2.1	100
6.	2.5	120
7.	2.9	140

Estimation of sample:

S.No	Solvent	Absorbance at 415nm	Total flavonoid(µg/mg)
1.	Aqueous	0.324	12.1

Antioxidant activity:

Free radical scavenging and the percentage inhibition of all the extracts against DPPH is presented in table 6 and 7. In that aqueous extract showed high free radical scavenging activity than other extracts.

Table 6. Absorbance of free radical from DPPH at 517nm

Blank: 0.000

S.No	Control	Sample	Optical Density					
			5’	10’	15’	20’	25’	30’
1.	0.977	BHT(0.16%)	0.597	0.444	0.336	0.256	0.202	0.170
2.	0.273	Sample A(hexane)	0.156	0.149	0.144	0.140	0.136	0.133
3.	0.273	Sample B(acetone)	0.139	0.124	0.115	0.102	0.099	0.095
4.	1.027	Sample C(aqueous)	0.310	0.305	0.301	0.298	0.293	0.288

Table 7. Percentage inhibition

S.No	Sample	% of Inhibition					
		5'	10'	15'	20'	25'	30'
1.	BHT(0.16%)	38.93	54.50	65.57	73.77	79.30	82.58
2.	Sample A(hexane)	42.85	45.42	47.25	48.71	50.18	51.28
3.	Sample B(acetone)	49.08	54.57	57.87	62.63	63.73	65.20
4.	Sample C(aqueous)	69.81	70.30	70.69	70.98	71.47	71.95

IV. CONCLUSION

The qualitative investigation of phytochemicals revealed the presence of flavonoids, alkaloids, cyanine, quinines and phenols in all the three extracts.

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