European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 7, Issue 10, 2020

PHYTOCHEMICAL ANALYSIS AND DPPH ANTIOXIDANT ACTIVITY OF ROOT AND BARK OF *SYZYGIUM STOCKSII* (DUTHIE) PLANT.

Shubhada S. Nayak[1], Gurumeet C Wadhawa [1] , Vitthal S. Shivankar[2], Inamadar R F [3]Maryappa C. Sonawale[3]

[1]Rayat Shikshan Sansthas, Karmaveer Bhaurao Patil College, Vashi sector 15 A Navi Mumbai, 400703, Maharashtra, India.,[2]Rayat Shikshan Sansthas, Chhatrapati Shivaji College, Satara, Maharashtra, India., [3] Department of Chemistry, Veer Wajekar A.S.C. College, Phunde, Raigad, Maharashtra.

Email: wadhava.gurumeet@gmail.com

ABSTRACT

The study is related to phytochemical analysis andexamination of antioxidant property of root with barkof Syzygiumstocksii(Duthie). Itoccurs in Ratnagiri region of the Maharashtra,India. Qualitative analysis of the phytochemicals was carried using known protocol and the in this we have calculated the phenolic and flavonoids present in plant by the protocol which are reportedin the reference. While the antioxidant activity was carried using the known (DPPH)assay. Thephytochemical constituent's and activitydiscoveredin root and bark or stem fraction of the plant shows the presence of the usual and natural compounds like carbohydrates, phenols, saponins, terpenoids, alkaloids, flavonoids and other components are also present in methanolic extract. The methanolic extract of the plant is richer than the water extract. The flavoid and phenolic compound with it show the ma maximum concentration as (2.129 milligram, 4.22milligram and lower in the water extract(548 milligram 624 milligram). There is positive correlation between phenol content and the DPPH activity. The root and bark of plant shows good antioxidant activity. The plant extract act as the good antioxidant agent for various supplement.

INTRODUCTION

The oxidation isa major cause of many diseases and disorders in animals. Most of the medicinal plants have good antioxidant activity by inhibiting free radicals and oxidation process. This

antioxidant present in plant, break free radical chain reactions. Various chemicals present in medicinal plant are accountable for the antioxidant action; and the chemicals with ascorbic acid with poplyphenolic compounds. They have to inhibit lipid peroxidation by inactivating lipoxygenase and responsible free radical by chelating with different metals.[1-2]various Hydrogen peroxide (H_2O_2) with superoxide anion (O_2),with different hydroxylradicals (OH^-) very active oxygen and various free radicals them. There various active free radicles are present which are present in human body and they mainly regulate the metabolic action and causes the important role in different diseases like heart diseases, cancer neurodegenerative diseases, and in the aging process. Plant are natural source of antioxidants. The ordinary antioxidants are flavonoids, phenolic acids, tannins alkaloids, lignin's, stilbenes. Natural antioxidants or other phytochemicals are well known free radicals, scavengers and different biological activity.[3]Various organic antioxidant were have the genotoxic effect[4]various chronic diseases.[5]Plants contain the glycosides, tannins alkaloids, protein, phenolic compound, glycosides saponin,steroid, reducing sugars.[6-7]Most of the plants contain flavonoids, steroids, alkaloids, lignin's, phytosterols, macronutrient analysis, different sugars , different reducing sugar, different fats presence of proteins, and oil.[8]

In scavenging assay by the DPPH IC 50 assessment was around 60 μg/mL was comparable with standard ascorbic acid.[9]For this we have used *Syzygiumstocksii* (Duthie) Gamble, belongs to family Myrtaceae(Myrtle family). *Syzygiumstocksii* is censoriously rare planttypes of IUCN with red list category and endemic to India. *Syzygiumstocksii* was published by Duthie as a *Eugenia stocksii* base on plant collection of J. E. stocks from Konkan region of Maharashtra in 1879. This species found only few localities of Konkan region of Maharashtra state, Uttara Kannada of Karnataka state, Wayanad district of Kerala state and Tamil Nadu state.[10-11] Trees, 10- 12 min

height , classic, baywith the colour greyish-brown in it and branches with Leaves simple, opposite; petiole *c.*with 1.5 cmin the long, with the stout, with different grooved at upper side , glabrous; lamina *c.* 12 × 8 cm, elliptic-oblong with erect with the base and narrowed, upper apex rounded or shortly with acute,margin entire, different cretaceous, rightly glabrous; there nerves 10–16 paired , finely dotted, paralleled , highly distant, prominent with the globular , different the warpedaloft and becoming weak towards the margin, not hitching into an intramarginal nerve, intercostalwith the reticulate, obscure in nature . Flowers are bisexual, they have small, abundant in pronged cymes which are axillary from the leafless axils; peduncle 2–5 cm, branches trim angled.[12-14]Calyx sections 4, funnel-shaped, having the rugose outside, smoothed. The Petals calyptrate, gland dotted. Stamens many, bent indoors at the mid when in bud. Berry inferior, 6 celled, style 1 with stigma simple. Fruit a berry,*c.* 0.7 × 8 cm, pink-purple,one seeded. Phenology- Flowering:[15] March–April; Fruiting: May–June.Voucherspecimen: ANC 1726Localities:GirmadeviKond, Rajapur tehsil of Ratnagiri District (Maharashtra).*Note:* This species can be easily identified by its tetragonousbranchlets, calyx tube turbinate; leaves membranous & more than 5 cm broad, leaf-nerves distant, inflorescence branches slender. After type collection this species first, time reported from Maharashtra. This species rarely found in evergreen forest along the margin of streams. *Lectotype:*India. Concan [Konkan], Stocks s.n. (K! [barcode K000260039]).

Fig. 1 Image of *Syzygiumstocksii* (Duthie)

MATERIAL AND METHODS

Selection of Plants:

GirmadeviKond, Rajapur tehsil of Ratnagiri DistrictMaharashtra, India,stood taken for experiment. These species of the plant authenticby Dr. Arun Chandore of AbasahebMarathe, Arts and New Commerce, Science College, Rajapur- 416702, Maharashtra, India. The different plant materials are together during monsoon.Bark and roots collected waswashing away with water by the several times,then shade dried for the 15 days. Thenthis material was dried in oven for 30^0C, till constant weight was obtained.Finally,ground into fine powder.This fine powder was kept in air tight container.

Preparation of the extract

The powder plant material extracted with methanol and water in 9:1 ratio by using the soxhlet apparatus for about 90 hrs. This was cooled at room temperature then filter through it was pass through the filter paper whatmannumber 1, then the remainder was the concentrate in vacuum

evaporator to dryness to form a fine powder. Powder dried till constant weight was obtainedand stored at room temperature.

Phytochemical Screening using Known Protocol

The different phytochemical present the water and methanol extract was carried for both the root and bark.[16-21]

Estimation of total phenol

Folin-cinocalteu assay using gallic acid was described for the phenolic content determination using the standard using the slight modification

Use Value (UV): value using UV data of different compoundshas high importance.They are locally calculated by UV = (ΣU/n), U speciesare reports as number and number of informants for given plant. 1mLof solution and folin-ciocalteu reagent (FCR) reagent 1 mL of 1 mL of 10% NaHCO3 and 8 mL distilled water gestated for 25 min.dark room using room temperature. The amount present in the plant the phenolic compounds are easilydone by simple spectrophotometry by gauging the absorbance by UV visible spectrophotometry using 780 nm in contradiction of blank water of 1 mL in tube with 1 mL of the 10 % sodium bicarbonate. Total phenol content was articulated in the Gallic acid equivalents in the given extract,this can be done using calibration curve of Gallic acid compound .there is linear calibration was shown as the 10 to 100μg/ml of the sample ($r = 0.99$).[22]

Estimation of the flavonoids

By using aluminum chloride spectrophotometric methods cast-off for the total flavonoid.[23]Total flavonoid[24-25]content was quercetin used as standard for determining the total flavonoid content.10% sample 100μl of $AlCl_3$, mixed with the 100μl sodium nitrate (5%), 670μl of 1

milimolar NaOH and 100µl in sample tube then gestated at temperature 25 ^{0}C and 520 nm was used for measurement.

Antioxidant Activity using the in vitro protocol

Radical scavenging assay DPPH method was used [26]powder roots and barkis dissolved in methanol as 9:1 and refluxed for around 90 hrs. Then cooled at 30^{0}C was subjected to rpm 20000 rpm.We prepareddifferent concentration by various sequentialweakeningmethods using methanol water used as various diluents and mixed with 0.16mM methanol and DPPH. Same protocol used for the ascorbic acid or vitamin C for the assessment and ration 3:1 mL mixture of methanol and DPPH as standard,water used as adverse control. Preoccupation was leisurely at 520 nm using ShimadzuUV visible spectrophotometer. The capability of scavenging DPPHin radicals[27-30]was calculated as

Scavenging effect % = [1-(optical density of sample /optical density of DPPH) X100

We have calculated The % scavenging effect is plotted by the logarithmic plot .

EC50 value (mg/µl) for 50 % extract of the plant and DPPH concentration

RESULT AND DISCUSSION

Plant was subjected to phytochemical analysis for both root and bark were reported in Table 1 and 2. Table 1 and 2 shows thequalitative phytochemical screening of water and organic extracts of root and barkof *Syzygiumstocksii* (Duthie) which shows alkaloids, phenol, flavonoids,tannins, saponins different carbohydrates. Table 1 and 2 also shows colour changes for the various phytochemicals , in methanol and water extracts.

Qualitative phytochemical screening of the Root

1] Trease and Evans, 2002 described the method for the detection of the Flavonoids using the Shinoda's test which gives red colour to methanol and pink colour water extract indicate the presence of the Flavonoids .this test was performed using the Few pieces of mg chips + few drops of con. HCl

2]Sofowora, 1993 describe the method for the detection of the Alkaloids known as the Dragendorff's Test performed using the 5mL 1% aqueous HCl + few drops of Dragendorff's reagent red colour to methanol and light red colour water extract is positive test for alkaloids

3] Trease and Evans, 2002 gives the test for the detection of the Phenols using the $FeCl_3$ Test has the 4mL of distilled water + a few drops of 10% $FeCl_3$ solution gives the presence of the phenol by blue colour to methanol extract and light blue colour to the aqueous extract

4] Trease and Evans, 2002 gives the method for the testing of the Tannin and using the $FeCl_3$ Test and 1% $FeCl_3$ solution for methanol extract gives Blue green ppt and the aqueous extract gives the Green ppt

5] Sofowora, 1993 used for the SaponinsFoam test in 3 mL of dist. H_2O it gives the foam in the methanol extract and no or less foam to the water extract

6] Sofowora, 1993 gives the test for the Carbohydrates we have used the Molisch's test by the α-Naphthol + 1mL conc. H_2SO_4 +5 ml dis H_2O it gives the red colour to the methanol extract indicate presence of the carbohydeate in the water extract no colour then carbohydrates are absent

Qualitative phytochemical screening of the Bark

1] Flavonoids are detected using the Trease and Evans, 2002 as the Shinoda's test using the Few pieces of mg chips + few drops of con. HCl methanol gives the Red and dark red indicate presence of the Flavonoids

2]Alkaloids are detected using Sofowora, 1993Dragendorff's Test by 5mL 1% aqueous HCl + few drops of Dragendorff's reagent it gives the red colour to the methanol extrac indicates these presence of alkaloids t and water extract no colour no presence of Alkaloids

3] Phenols are detected Trease and Evans, 2002 by using $FeCl_3$ Test as the 4mL of distilled water + a few drops of 10% $FeCl_3$ solution for the presence of the methanol extract gives the blue colour and water extract gives the Dark blue for the blue.

4] Trease and Evans, 2002 test for Tannin for the $FeCl_3$ Test as the 1% $FeCl_3$ solutionmethnol extract Blue green ppt gives the Dark Green ppt indicate the presence of saponnins

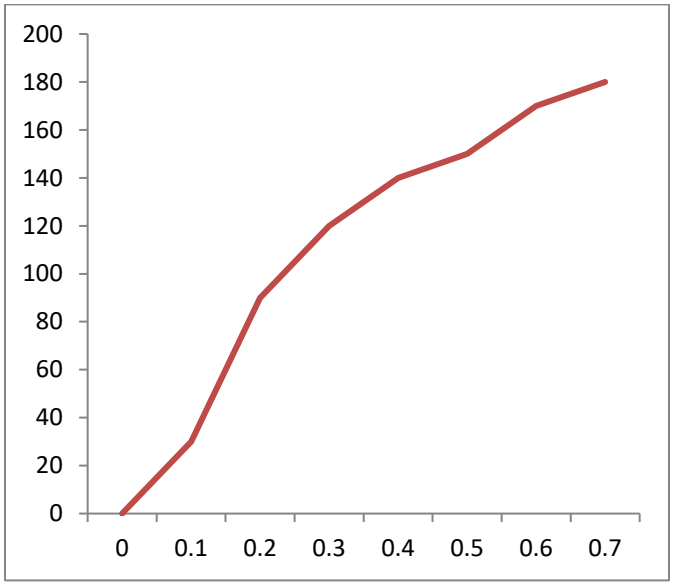
5]Sofowora, 1993 gives the test Carbohydrates asMolisch's testα-Naphthol + 1mL conc. H_2SO_4 +5 mL dist. H_2O gives the methanol extract Red colour and no colour for the aqueous extract

Folin-Ciocalteu Reagent (FCR) Method for the detection of the phenol

Most of time the for the phenolic compounds having the OH radicals,these having the foraging activity and work as the with the antioxidants. Total phenolic concentration was used for the quick scanning of the antioxidant activity.The total phenolic content was articulated as gallic acid equivalent (mg of GAE/gm sample) byequation based on the calibration curveY = 0.007 X - 0.036 R2=0.997

While X axis there was absorbance

Yaxis indicate the GAE/gm sample.



Conc	Absorbance
0	0
0.1	30
0.2	90
0.3	120
0.4	140
0.5	150
0.6	170
0.7	180

Gallic acid Curve

The total phenolic content plant was

phenolic content for plant

Root for Methanol Water the total phenol content in mg 2.45 and 2.30.*Bark Methanol* Water total phenol content 1.40 and 1.25

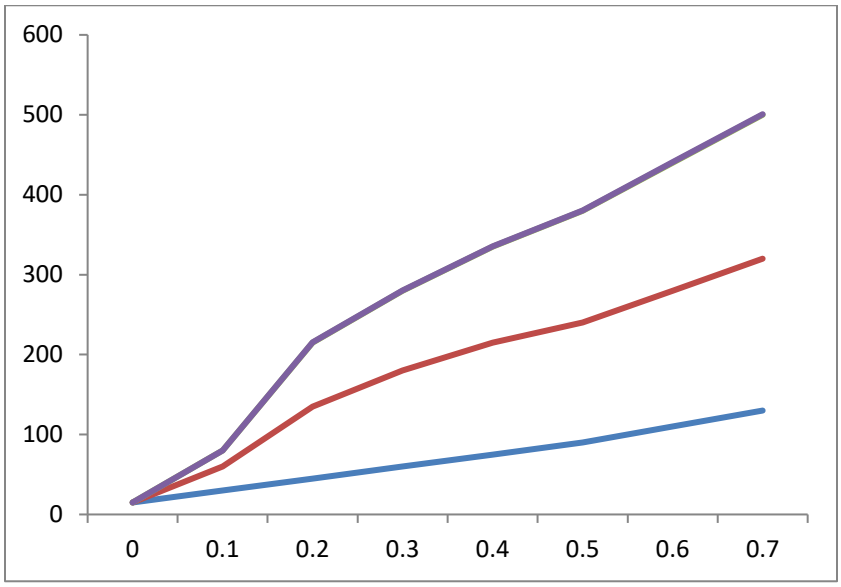
Total Flavonoid Content flavonoid mostly contains the flavones, flavanols and condensed tannins having the hydroxy **groups**

Root contain the Methanol Water Flavonoid content 4.22.3.89 Bark Methanol Water having the Flavonoid content1.125 and 1.56

DPPH Antioxidant Activity:

Yellow colour was obtained when powder(antioxidant) was added to the DPPH. the antioxidant activity and antioxidant capacity of extract [EC 50] was compared with the ascorbic acid as positive control

Ascorbic acid Violet Line	Root Red Line	Bark Blue Line	conc
0	0	0	0
30	30	20	0.1
45	90	80	0.2
60	120	100	0.3
75	140	120	0.4
90	150	140	0.5
110	170	160	0.6
130	190	180	0.7



EC50 Value:

The EC 50 values are inversely related to the antioxidant activity

The DPPH radicle scavenging activity is usually quantified in the term of the inhibition percentage of the preformed free radicles by the antioxidants while the EC(50) (concentration required to obtain a 50% antioxidant effect) is used to express the antioxidant capacity compare to other extract's

CONCLUSION:

We have carried out the various phytochemical test of the methanoloic and aqous extract of *Syzygiumstocksii* (Duthie) was studied it has indicate that it contain the various phytochemicals such as the alkaloids, carbohydratessaponins,phenols,tannins,flavonoid are present in both the extracts with their reagents .the root parts of the plant shows the highest activity as compare to the bark .the of plant *Syzygiumstocksii* (Duthie) act as good antioxidating agent .these is good correlation between the total phenolic content and the antioxidant capacity.this has been suggest that the both root and bark of the plant have very good theraptic agent and used for the preventing the ageing and oxidative stress .this plant can act as good antioxidant activity.

Table5: EC50 VALUE

Plant Part	**EC50 value (µg/ml)**
Root	38.06
Bark	32.43

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS:

We are thankful to Principal of the collegefor Research Promotion and ethics committee, Rayat Shikshan Sanstha Satara for help.

REFERENCES

1. Muley BP, Khadabadi SS, Banarase NB, Sawarkar HA. The Antioxidant Activity of the Leaves and Petals of Calendula officinalis Linn. Research J. Pharm. and Tech.,2009;2(1): 173-175.

2. Shinde N, Saindane RA, Bhosale SK, Deshpande S. In Vitro Antioxidant Activity of Ethanolic and Chloroform Extracts of Tageteserecta Flowers. Research J. Pharmacognosy and Phytochemistry, 2010; 2(4): 324-326.

3.Pareta SK, Patra KC, Mazumder PM, Sasmal D, *et al*. Establishing the Principle of Herbal Therapy for Antiurolithiatic Activity: A Review J PharmacolToxicol. 2011;6(3):321-323.

4. Adepu A, Narala S, Ganji A, Chilvalvar S. A Review on Natural Plant: *Aervalanata*. Int. J. Pharma. Sci. 2011;3(6):398-402.

5.Venugopal SN. Herbal stone crushers; The *Pashanabheda*. Medplant-Envis Newsletter on medicinal plants. 2009;1(2):6-9.

6. Catherine CI, Jude CI, Edward OA, Eugene NO. Quantitative Determination of Alkaloid, Allicin, Glycoside and Saponin Constituents of the Leaves of Sansevieriasenegambica Baker by Gas Chromatography. Research J. Science and Tech. 2011; 3(6): 308-312.

7. Muthupandi VA and Sundaram SR: Phytochemical Evaluation and *in vitro* Antioxidant Activity of Various Solvent Extracts of *Leucasaspera*(Willd.) Link Leaves. Free Radicals and Antioxidants 2017; 7(2): 166-171.

8. Kumar MS, Balachandran S, Chaudhury S. Influence of Incubation Temperatures on Total Phenolic, Flavonoids Content and Free Radical Scavenging Activity of Callus from Heliotropiumindicum L. Asian J. Pharm. Res. 2012; 2(4): 148-152.

9. Brand-Williams W, Cuvelier ME, Berset CL. Use of free radical method to evaluate antioxidant activity. LebensmWiss Technology. 1995;28(1):25-30.

10. Cooke, T. The Flora of the Presidency of Bombay. Vol. 1, Taylor & Francis, London. 1905; Pp. 491.

11. Hooker,JD. (1880): Flora of British India Vol. 2, L. Reeve & Co, London. Pp. 498.

12. http://specimens.kew.org/herbarium/K000260039

14. IUCN (2020) https://www.iucnredlist.org/species/31201/9611615

15. Singh, N.P., P. Lakshminarasimhan, S. Karthikeyan & P. Prasanna (2001): Flora of Maharashtra State. Dicotyledones Vol. 2, Botanical Survey of India, Kolkatta. Pp. 15.

16. Trease GE and Evans WC: Pharmacognosy. 15th Ed. London: Saunders Publishers 2002; 42–44. 221–229, 246–249, 304–306, 331–332, 391–393.

17. Sofowora A: Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Sunshine House, Ibadan, Nigeria: Spectrum Books Ltd. Screening Plants for Bioactive Agents 1993; 134–156.

18. Rajendran R, Hemachander R, Ezhilarasan T, Keerthana C, Saroja DL, Saichand KV, Abdullah MG. Phytochemical Analysis and In-Vitro Antioxidant Activity of Mimosa pudica Lin., Leaves. Research J. Pharm. and Tech. 2010; 3(2): 551-555.

19. Srivastava S, Jaiswal J, Gautam H, Singh S, Sharma S. Phytochemical Analysis of Leaves of Hibiscus rosa-sinensis. Research J. Pharmacognosy and Phytochemistry 2013; 5(4):182-184.

20. Patil PJ, Patil VR. Phytochemical and Toxicological Evaluation of Acoruscalamus and Argyreiaspeciosa Leaves Extract. Res. J. Pharmacognosy and Phytochem. 2016; 8(3): 121-124.

21. Velraj M, Armstrong, Ravichandran, Jeyakumari, Hemalatha, Vijayalakshmi, Srikanth.J. Pharmacognostical and Preliminary Phytochemical Studies on the Stem Bark of Soymidafebrifuga (Roxb). Research J. Pharmacognosy and Phytochemistry 2009; 1(3): 213-216.

22. Marinova G, Batchvarov V. Evaluation of the methods for determination of the free radical scavenging activity by DPPH. BULG J AGRIC SCI. 2011;17(1):11-24.

23. Brand-Williams W, Cuvelier ME, Berset CL. Use of free radical method to evaluate antioxidant activity. LebensmWiss Technology. 1995;28(1):25-30.

24. Wadkar KA, Magdum CS. Evaluation of Total Phenolic Content, Flavonoid Content and Antioxidant Activity of Stem Bark of CareyaarboreaRoxb. Research J. Pharmacognosy and Phytochemistry 2010; 2(1): 49-51.

25. Gopalasatheeskumar K, Ariharasiva Kumar G, Sengottuvel T, SanishDevan V, Srividhya V. Quantification of Total Phenolic and Flavonoid content in leaves of Cucumismelovaragrestis using UV- spectrophotometer. Asian J. Research Chem. 2019; 12(6):335-337.

26. McDonald S, Prenzler DP and Antolovich M: Phenolic content and antioxidant activity of olive extracts. Food Chem., 2001; 73: 73–84.

27.Jadhav GB, Saudagar RB. Free radical Scavenging and Antioxidant Activity of Punicagranatum Linn. Asian J. Res. Pharm. Sci. 2014; 4(2): 51-54.

28. Munne S, Parwate DV, Ingle VN, Panchbhai DS, Nagpurkar VS. Free Radical Scavenging Activity of Gamma Irradiated and Unirradiated Citrus medica Empty Juice Sacs. Asian J. Research Chem. 2011; 4(6): 957-959.

29. SheelaS, Babu ND, Ilango K. Free Radical Scavenging Activity of Leaves of MemecyloneduleRoxb. Research J. Pharmacognosy and Phytochemistry 2009; 1(2): 109-112.

30. Balamurugan G, Arunkumar MP, Muthusamy P, Anbazhagan S. Preliminary Phytochemical Screening, Free radical Scavenging and Antimicrobial activities of Justiciatranquebariensis Linn. Research J. Pharm. and Tech. 2008; 1(2): 116-118.