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Original Article

# A Study on Antioxidant Activity of Three Ethanolic Extract Polyherbal Formulation

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ABSTRACT

The present work Carried out the anti oxidant activity of different polyherbal formulations containg different portion of ethanolic extract. Based on litreture survey it has selected traditional used three *plants Polygonum glabrum, Canthium dicoccum, Ochna obtusata,* collected and plant material is dried according to the standard procedure and using ethanol as solvent the plants are extracted individually The extract is dried and prepared polyherbal formulation of the ethanol extract and used In vitro analysis of anti-oxidant activity. In the different polyherbal formulations of ethanolic extract F1 and F2 having the high flavonoid contents and anti-oxidant activity when they tested by *In-vitro* methods. Among those two formulations F2 having the high flavonoid content and this can be used for futher evaluation for pharmacological activities.

**Key words:** polyherbal formulation, antioxidant activity, *Polygonum glabrum, Canthium dicoccum, Ochna obtusata,* DPPH, NO-scavenging, flavonoid.

## 1. INTRODUCTION

Antioxidants are involved in the defense mechanism of the organism against the pathologies associated to the attack of free radicals. Endogenous antioxidants are enzymes, like superoxide dismutase, catalase, glutathione peroxidase or nonenzymatic compounds, such as uric acid, bilirubin, albumin, metallothioneins. When endogenous factors cannot ensure a rigurous control and a complete protection of the organism against the reactive oxygen species, the need for exogenous antioxidants arises, as nutritional supplements or pharmaceutical products, which contain as active principle

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an antioxidant compound. Amongst the most important exogenous antioxidants, vitamin E, vitamin C, β-carotene, vitamin E, flavonoids, mineral Se are well known, but also vitamin D and vitamin K3. Exogenous antioxidants can derive from natural sources (vitamins, flavonoids, anthocyanins, some mineral compounds), but can also be synthetic compounds, like butylhydroxyanisole, butylhydroxytoluene, gallates, etc [1]. There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, as well as the deterioration of fats and other constituents of foodstuffs [2]. Recently, antioxidants have attracted considerable attention in relation to radicals and oxidative stress, cancer prophylaxis and therapy, and longevity [3]. Phenols and polyphenols are the target analytes in many such cases; they may be detected by enzymes like tyrosinase or other phenol oxidases, or even by plant tissues containing these enzymes [4-18].

***Polygonum glabrum*** which is commonly called as dense flower knotweed is a semi aquatic perennial plant. It belongs to the family polygonaceae and genus polygonum [19]. This particular genus consists of more than hundred species out of which nearly seventy are present in marshy lands of India. Family polygonaceae consists of large number of medicinal plants and is well known for its use in ethnomedicine. The glabrum species of the genus polygonum provide a variety of traditional properties. The tribes of chattisgarh use the root paste as a medicine for snake bite [20]. In some areas the root stock is used for the treatment of jaundice and piles [21]. The leaves are used as an antimalarial agent in sudan [22]. In south india, *Polygonum glabrum* leaves are used for the treatment of dysentery [23]. A decoction of the leaves and seeds are used as cardiotonic, astringent and anthelmintic [24].The whole plant decoction is used as a remedy for colic pain, pneumonia and the boiled paste is applied in cuts and wounds [25]. Apart from medicinal use, the whole plant is powdered and used as bait for fishing. Peels from stem are used for treating rheumatism [26].

***Ochna obtusata* DC** (Family-Ochnaceae). Habit: Small trees up to 8 m tall. Trunk & Bark: Bark greyish, smooth; blaze pinkish. Branches and branchlets: Branchletsterete, lenticellate, glabrous. Leaves : Leaves simple, alternate, distichous; stipules caducous and leaving scar; petioles ca. 0.4 cm long, planoconvex, glabrous; lamina 16 x 5 cm, elliptic or elliptic-oblong to obovate, apex acute to rounded, base acute to rounded, margin serrate, shining above, chartaceous, glabrous beneath; midrib raised above; secondary_nerves ca. 12 pairs, ascending towards apex; tertiary_nerves slender, reticulo-percurrent. Inflorescence / Flower: Inflorescence axillary or lateral racemes; flowers yellow; pedicels up to 2.5 cm long. Fruit and Seed: Drupe, 3-5 distinct drupes seated on the enlarged disk; seeds 1 drupe. Distribution: South Asia; in the Western_Ghats- South, Central and Maharashtra Sahyadris. The leaves and roots of *Ochna obtusata* is used for ulcer, asthma and bronchitis [27-29]. From the source of literature documentation and relevant traditional approaches on plant drugs,

***Canthium dicoccum*** also known as nallabalusu (telugu), nallamandharam (tamil) in India belongs to the family Rubiaceae. The plant is found in deccan peninsula, maharastra southwards, and extending from bihar eastwards to assam and Meghalaya. The plant is a smooth shrub 3 to 4 meters or more in height. Leaves are extremely variable, ovate, elliptic, ovate or somewhat rounded, 5 to 15 centimeters long, 1.5 to 10 centimeters wide, leathery, shining above, and usually pointed at both ends. Flowers are white, with very slender stalks, 5 to 10 millimeters long, and borne in compressed, short-stalked cymes. Calyx is cut off at the end or obscurely toothed. Corolla is bell-shaped, with a 4- to 6-millimeter tube, and five somewhat pointed lobes. Fruit is rounded, ellipsoid or obovoid, 6 to 10 millimeters long, slightly flattened and obscurely 2-lobed. In India, bark is used for fever. Decoction of roots used for diarrhea it contains a new flavonol glycoside, 7-O-(5-O-benzoyl-ß-D-glucopyranosyl)-rutin [30]. Diglycosides, rutin and its benzoic derivative, 7-O-(5-O-benzoyl-ß-D-glucopyranosyl)-rutinfrom C dicoccum and kaempferol 3-ß-D-rutinoside from C rheedii strongly inhibited all test fungi. [31] ethanolic extract of whole plant of Canthiumdiococcum for anti-inflammatory activity in Wistar albino rats in various models of anti-inflammatory activity viz. Carrageenan induced paw edema, Formalin induced paw edema, fresh egg white induced paw edema and cotton pellet induced granuloma model. Results showed the extract with anti-inflammatory activity and suggests a potential alternative to NSAIDS like diclofenac [32, 33].The ethanolic extract of *Canthium diococcum* for anti-diabetic in an alloxan induced diabetic rat model showed a significant drop in fasting blood sugar in a dose-dependent manner, with an effect on the beta-cell population in the pancreas. The extract showed almost equipotent antidiabetic activity compared to standard drug Glibenclamide [34].Ethanolic extract for anti-arthritic activity in albino rats. Results showed significant anti-arthritic activity against Egg-albumin induced arthritis model [35]. The ethanolic extract of leaf yielded major chemical constituents viz. Spathulenol (20.76 %), Caryophyllene oxide (19.25 %), Cedren-13-ol (10.62 %), Ledene oxide (5.24 %), m-mentho-4, 8-diene (6.41 %) and 2-furancarboxaldehyde (4.51 %). Some on the constituents provide scientific bases and evidence for antimicrobial, anti-tumor, immunomodulatory, and antioxidant properties of the plant [36].

## 2. MATERALS AND METHOD

### Plant source and authentication

*Polygonum glabrum, Ochna obtusata DC and Canthium dicoccum* were collected from Tirumala Hills, Tirupati, and Chittoor district of Andhra Pradesh, near Seshachalam and Tirumala Hills (Rayalaseema region, Andhra Pradesh, India), areas that are geographically located in the South

Eastern Ghats, are recognized for their rich flora and fauna [48]. The plant specimen was verified to be of the correct species by Dr. Madhava Setty, a botanist from the Department of Botany, S. V. University,Tirupati.

**Chemicals**

Freund's adjuvant complete (CFA), N-methoxysuccinylAla-Ala-Pro-Val p-nitroanilide and Griess Reagent system were purchased from Sigma Chemical Co. (St Louis, MO, USA). Collagen type II from bovine nasal septum was purchased from Elastin Products Co, INC, Owensville, Missouri, USA. Thiobarbituric acid (TBA), trichloroacetic acid (TCA), 5-5'dithio-bis-2-nitrobenzoicacid (DTNB), Nitrobluetetrazolium (NBT), ethylene diamine tetra-acetic acid (EDTA), xanthine, xanthine oxidase, Tris hydrochloride were purchased from SD Fine chemicals

India. All other routine chemicals used in this investigation were of research grade.

**Preparation of poly herbal extract**

Aerial parts of *Polygonum glabrum, Canthium dicoccum, Ochna obtusata*, were collected and dried. Then the material was blended to form a fine powderand extracted ethanol using soxhlet apparatus for 6 hrs at 50ºC and water by maceration The solvent was completely removed by rotary evaporator (Rotavapor® R-210, BUCHI Corporation) and respective extracts preserved for various investigations.

**Preparation of polyherbal formulations using crude extracts.**

The above extract used for the preparation of five different poly herbal formulations with varying proportions and working formula given in the table.1

***In vitro* anti-oxidant activity of poly herbal formulation**

**DPPH Radical Scavenging Assay**

The DPPH assay method is based on the reduction of DPPH, a stable free radical [48]. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). When Antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical-scavenging antioxidant) and is reduced to the DPPHH and as consequence the absorbance's decreased from the DPPH. Radical to the DPPH-H form results in decolorization (yellow colour) with respect to the number of electrons captured. More the decolorization more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Diphenylpicrylhydrazine; non radical) with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present. The scavenging reaction between (DPPH.) and an antioxidant (H-A) was shown in figure 2. 4.3 mg of DPPH (1, 1-Diphenyl –2-picrylhydrazyl) was dissolved in 3.3 ml methanol; it was protected from light by covering the test tubes with aluminum foil. 150 µl DPPH solution was added to 3ml methanol and absorbance was taken immediately at 517nm for control reading. 50 µl of various concentrations of coumarin compounds as well as standard compound (Ascorbic acid) were taken and the volume was made uniformly to 150 µl using methanol. Each of the samples was then further diluted with methanol up to 3ml and to each 150 µl DPPH was added. Absorbance was taken after 15 min. at 517nm using methanol as blank on UV-visible spectrometer Shimadzu, UV-1601, Japan. The IC50 values for each drug compounds as well as standard preparation were calculated. The DPPH free radical scavenging activity was calculated using the following formula:

% scavenging = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100…(Equation 1)

**Nitric oxide free radical scavenging activity**

Nitric oxide (NO) has also been involved in a variety of biological functions, including neurotransmission, vascular homeostasis, antimicrobial, and antitumor activities. Despite the possible beneficial effects of NO its contribution to oxidative damage is also reported. This is due to the fact that NO can react with superoxide to form the peroxynitrite anion, which is a potential oxidant that can decompose to produce OH and NO. The procedure is based on the principle that, sodium nitro-prusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent [52-54]. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. Large amounts of NO may lead to tissue damage. 50 µl of each of the concentrations of coumarin compounds previously dissolved in DMSO, as well as ascorbic acid (standard compound) were taken in separate tubes and the volume was uniformly made up to 150 µl with methanol. To each tube 2.0 ml of sodium nitroprusside (10 mM) in phosphate buffer saline was added. The solutions were incubated at room temperature for 150 minutes. The similar procedure was repeated with methanol as blank which served as control. After the incubation, 5 ml of griess reagent was added to each tube including control. The absorbance of chromophore formed was measured at 546 nm on UV-visible spectrometer Shimadzu, UV-1601, Japan. Ascorbic acid was used as positive control. The IC50 values for each test compounds as well as standard preparation were calculated.

% scavenging = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100…….(Equation-2)

**Estimation of total flavonoid content**

Total flavonoid content was determined by aluminium chloride method. 0.5 ml of the extract was mixed with 1.5 ml methanol, 0.1 ml 10 % AlCl3, 0.1 ml of 1M potassium acetate and 2.5 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. All determinations were carried out in triplicates. Using Rutin, standard curve was prepared and linearity was obtained in the range of 1-10

µg/ml. The total flavonoid content was expressed as rutin equivalent in mg/g of the extract [50].

**Estimation of total phenol content**

In a test tube 200 µl of the extract (1 mg/ml to 0.1 mg/ml) was mixed with 1 ml of Folin-Ciocalteu reagent and 800 µl of sodium carbonate. After shaking, it was kept for 2 h reaction time. The absorbance was measured at 750 nm. Using gallic acid monohydrate, standard curve was prepared and linearity was obtained in the range of 0.78-25 µg/ml. Using the standard curve the total phenol content was obtained. All measurements were carried out in triplicates. The total phenol content was expressed as gallic acid equivalent in mg/g of the extract [49].

## 3. RESULTS

**Table 1: Different types of formulations using Ethanolic extracts of four different plants**

| S NO | Different ratios of ethanolic extract of three plants | | |
|---|---|---|---|
| | *Polygonum glabrum* | *Canthium dicoccum* | *Ochnaobtusata* |
| FORMULATION I (F1) | 1 | 1 | 1 |
| FORMULATION II (F2) | 2 | 1 | 1 |
| FORMULATION III (F3) | 1 | 2 | 1 |
| FORMULATION IV (F4) | 1 | 1 | 2 |

**Table 2: DPPH Radical Scavenging Assay for crude ethanolic extract of different poly herbal formulations**

| S. No | Concentration(µg/ml) | Formulations | | | |
|---|---|---|---|---|---|
| | | F1 | F2 | F3 | F4 |
| 1 | 10 | 8.4 | 10.6 | 10.8 | 10.6 |
| 2 | 20 | 16.6 | 23.6 | 22.6 | 23.6 |
| 3 | 40 | 35.8 | 43.8 | 43.6 | 43.8 |
| 4 | 60 | 56.6 | 61.2 | 61.2 | 61.6 |
| 5 | 80 | 76.6 | 82.3 | 81.4 | 81.8 |
| 6 | 100 | 89.8 | 98.6 | 98.2 | 99.6 |
| 7 | IC50 | 54.5 | 56.9 | 48.6 | 52.08 |

**Table 3: Nitric oxide free radical scavenging activity for crude ethanolic extract of different poly herbal formulations**

| S.No | Concentration(µg/ml) | Formulations | | | |
|---|---|---|---|---|---|
| | | F1 | F2 | F3 | F4 |
| 1 | 10 | 9.4 | 9.6 | 10.8 | 8.6 |
| 2 | 20 | 19.6 | 23.6 | 22.6 | 19.21 |
| 3 | 40 | 39.8 | 41.8 | 43.6 | 41.8 |
| 4 | 60 | 61.2 | 62.2 | 59.2 | 57.4 |
| 5 | 80 | 78.2 | 82.3 | 79.4 | 73.2 |
| 6 | 100 | 92.4 | 96.6 | 92.2 | 82.6 |
| 7 | IC50 | 51.5 | 58.8 | 50.2 | 54.1 |

**Table 4: Total phenolic and flavonoid contents of Ethanolic extract of polyherbal formulations**

| S.No | Formulations | Total phenolic content of Ethanolic extract | Total flavanoid content of Ethanolic extract |
|---|---|---|---|
| 1 | F1 | 9.13±0.554 | 0.166 |
| 2 | F2 | 16.26±0.554 | 2.453 |
| 3 | F3 | 10.33±0.042 | 1.232 |
| 4 | F4 | 12.52±0.068 | 1.652 |

## 4. DISCUSSION

The present experimental procedure used for evalution of in vitro anti oxidant activity of different formulations (F1,F2,F3.F4, IN Table no 1).Single method is not suitable and could not judge the anti-oxidant activity hence here two method DPPH(Table no 2) and NO(Table no 3)used for the determination of anti-oxidant activity. Among the formulations formulation F2 and F4 having the higher. Ic 50 values respectively 58.8 and 54.1 and The Total Phenolic and flavonoid content are rich in the F2 formulation when compared with standard.

## 5. CONCLUSION

From this result Poly herbal formulation F2 having *Polygonum glabrum*, *Canthium dicoccum*, *Ochna obtusata* respectively in the ratio of 2, 1, and 1 is having the better antioxidant activity and flavonoid content. Hence this polyherbal formulation of ethanoli extract can be used for further studies to know the specific pharmacological activities.

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## 7. REFERENCES

1. Litescu SC, Eremia SA, Diaconu M, Tache A, Radu GL. Biosensors applications on assessment of reactive oxygen species and antioxidants. Environmental Biosensors. 2011:95.
2. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 2004;26:211-9.
3. Kalcher K, Svancara I, Buzuk M, Vytras K, Walcarius A. Electrochemical sensors and biosensors based on heterogeneous carbon materials. Monatshefte für Chemie-Chemical Monthly. 2009;140:861-89.
4. Ly SY. Voltammetric analysis of DL-α-tocopherol with a paste electrode. J Sci Food Agric 2008;88:1272-6.
5. Kong YT, Imabayashi SI, Kano K, Ikeda T and Kakiuchi T. Peroxidasebasedamperometric sensor for the determination of total phenols using twostage peroxidase reactions. Am J Enol Vitic 2001; 52: 381-5.

6. Mena ML, Carralero V, Gonzalez-Cortes A, Yanez-Sedeno P, Pingarron JM. Bioelectrochemical evaluation of the total phenols content in olive oil mill wastewaters using a tyrosinase–colloidal gold–graphite–Teflon biosensor. Int J Environ Anal Chem 2007; 87: 57-65.
7. Granero AM, Fernandez H, Agostini E and Zon MA. Anamperometric biosensor for trans-resveratrol determination in aqueous solutions by means of carbon paste electrodes modified with peroxidase basic isoenzymes from brassica napus. Electroanalysis 2008; 20: 858-64.
8. Zoulis NE and Efstathiou CE. Preconcentration at a carbon-paste electrode and determination byadsorptive-stripping voltammetry of rutin and other flavonoids. Anal Chim Acta 1996; 320: 255-61.
9. Volikakis GJ, Efstathiou CE. Stripping voltammetry using nujolgraphite and diphenylether-graphite paste electrodes. Talanta 2000; 51: 775-85.
10. Korbut O, Buckova M, Labuda J, Gruendler P Voltammetric detection of antioxidative properties of flavonoids using electrically heated DNA modified carbon paste electrode. Sensors 2003; 3: 1-18.
11. Cummings EA, Mailley P, Linquette-Mailley S, Eggins BR, McAdams ET, et al. Amperometric carbon paste biosensor based on plant tissue for the determination of total flavanol content in beers. Analyst 1998; 123: 1975-80.
12. Eggins BR, Hickey C, Toft SA and Zhou DM Determination of flavonols in beers with tissue biosensors. Anal Chim Acta 1997; 347: 281-288.
13. Cummings EA, Linquette-Mailley SC, Mailley P, Cosnier S, Eggins BR, et al. A comparison of amperometric screen printed carbon electrodes and their application to the analysis of phenolic compounds in beers. Talanta 2001; 55: 1015-1027.
14. Busch JLHC, Hrncirik K, Bulukin E, Boucon C, Mascini M . Biosensor measurements of polar phenolics for the assessment of the bitterness and pungency of virgin olive oil. J Agric Food Chem 2006; 54: 4371-4377.
15. Labuda J, Buckova M, Heilerova L, Caniova-Ziakova A, Brandsteterova E et al. Detection of Antioxidative Activity of Plant Extracts at the DNA-Modified Screen-Printed Electrode. Sensors 2002; 2: 1-10.
16. Kim HJ, Chang SC and Shim YB. Cyclodextrin modified screen printed graphite electrodes for detection of phenols. Bull Korean Chem Soc 2002; 23: 427- 431.
17. Capannesi C, Palchetti I, Mascini M and Parenti A Electrochemical sensor and biosensor for polyphenols detection in olive oils. Food Chem 2000; 71: 553-562.
18. Romani A, Minunni M, Mulinacci N, Pinelli P, Vincieri FF, et al. Comparison among differential pulse voltammetry, amperometric biosensor, and HPLC/DAD analysis for polyphenol determination. J Agric Food Chem 2000; 48: 1197-1203.
19. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. First ed. CSIR, New Delhi.1956; 199200.
20. Kadel C, Jain AK. Folklore claims on snakebite among some tribal communities of Central India. Indian J Tradit Know 2008; 7:296-9.
21. Shiddamallayya N, AzraYasmeen, Gopakumar K. Medico-botanical survey of kumarparvathakukkesubramanya, Mangalore, Karnataka. Indian J Tradit Know 2010; 9:96-9.
22. El Tahir A, Satti GM, Khalid S A. Antiplasmodial activity of selected Sudanese medicinal plants with emphasis on maytenussenegalemsis. J Ethnopharmacol 1999; 64: 227-33.
23. Soudahmini E, Ganesh M, Senthil PL, Madhu C, Divakar. Herbal remedies of Madugga tribes of Siruvani forest, South India. Nat Prod Rad 2005; 4: 492-9.
24. Shankar LH, Mishra PK. Study of aquatic medicinal plants of Hazaribagh district of Jharkhand, India. Int Res J Pharm 2012; 3: 405-9.
25. Koche DK, Shirsat RP, Syed Imran, Mohd. Nafees, Zingare AK, Donode KA. Ethnomedicinal Survey of nagzira wild life sanctuary, District Gondia (M.S.) India-Part II. Ethnomedicinal Leaflets 2008; 1(8): 532-7.
26. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary, Springer Science & Business Media, LLC, NY, USA. 2007: 509.
27. MadhavaChetty K. Yucca gloriosa Linn. Chittoor medicinal plants, Himalaya Book Publications, Tirupati, 2005, pp 60.
28. Sasidharan, Biodiversity documentation for Kerala-Flowering Plants, part 6: 85. 2004;
29. Ravikumar K, Sankar RV, Ved DK, Bhat KG. Is Madhuca insignis (Radlk) HJ Lam (Sapotaceae) really extinct. Phytotaxonomy. 2004;4:119-23.
30. Gunasegaran R, Subramani K, Parimala PA, Nair AR, Rodriguez B, Madhusudanan KP. 7-O-(6-O-Benzoyl-β-d-glucopyranosyl)-rutin from leaves of Canthium dicoccum. Fitoterapia 2001;72:201-5.
31. Vuyyuri B. Anti-Inflammatory Activity of Ethanolic Extract of Canthium dicoccum. Int J Pharm Phytopharmacol Res 2013; 3: 226-30.
32. Karunyal JS and Andrews B "Traditional medicinal plant wealth of Pachalur and Periyur hamlets Dindigul district, Tamilnadu. Indian J Tradit Know 2010, 9: 264-70.
33. Raja Rajeswari N, RamaLakshmi S, Muthuchelian K. GC-MS Analysis of bioactive components from the ethanolic leaf extract of Canthium dicoccum (Gaertn.) Teijsm&Binn. J Chem Pharm Res 2011; 3(3): 792-8.
34. Patel PD, Patel NJ, Patel DD, Patel RK. In-Vivo evaluation of Pleurotus sajorcaju mycelium extract for

Anti-inflammatory activity. Pharmacologyonline 2011;2:784-9.
35. Asim KG, Manasi B, Ghosh AK, Banerjee M, Bhattacharyya NK. Anti-inflammatory activity of root of Alpinia galanga Wild. Chron Young Sci 2011;2:139-43.
36. Kumar V, Abbas KA, Fausto N, Aster CJ Robins and Cotran: Pathologic basis of disease, 8 th edition, Sauders Elsevier, Philadelphia, 2010, 43.
37. Singh MP and Panda. H. Medicinal Herbs with Their Formulations Volume I, Daya Publishing House Delhi 2005; p: 115-116
38. Miller MD. Isolation and Identification of Lysergic acid and isolysergic acid as the principle ergoline alakoidsin Argyreia nervosa, a tropical wood rose. Association of analytiacal Chemist, 1970; 53:123-7.
39. Chao JM, Der Marderosian AH. Ergoline alkaloidal constituents of Hawaiian baby wood rose, Argyreia nervosa (Burm. f.) Bojer. J Pharm Sci 1973;62:588-91.
40. Agarwal SK, Rastogi RP. Ergometrineandother constituents of Argyeiaspeciosa. Ind J Pharmacol 1974;36:118-9.
41. Petra Mann, Britta Tofern, MackiKaloga and EckartEich. Flavonoid sulfates from the Convolvulaceae. Phytochemistry 1999;50:267-71.
42. YNShukla, Anil Srivastav, Sunil Kumar, Sushil Kumar. Phytotoxic activity and antimicrobial constituents of Argyreiaspeciosa and Oentherabiennis. J Ethnolpharmacol 1999; 67:241-5.
43. RahmanA, Ali M, Khan NZ. Argyroside from Argyeia nervosa seeds. Pharmazie 2003; 58: 60-62.
44. Mishra SH, Chaturvedi SC. Antibacterial and antifungal activity of the oil and unsaponifiable matter of Argyreia speciosa sweet. Indian Drugs and Pharmaceuticals 1978;13:29-31.
45. GokhlaeAB, Damre AS, Saraf HN. Investigations into the immunomodulatory activity of Argyreia nervosa. J Ethnopharmacol 2003; 84:109-14
46. Shaw Cross WE. Recreational use of ergoline alkaloids from Argyreia nervosa. J Psychoact Drugs 1983;15:251-259.
47. Kaur C, Kapoor HC. Anti-oxidant activity and total phenolic content of some Asian vegetables. International Journal of Food Science & Technology 2002 ;37:153-61.
48. Kasetti RB, Rajasekhar MD, Kondeti VK, Fatima SS, Kumar EG, Swapna S, Ramesh B, Rao CA. Antihyperglycemic and antihyperlipidemic activities of methanol: water (4: 1) fraction isolated from aqueous extract of Syzygium alternifolium seeds in streptozotocin induced diabetic rats. Food Chem Toxicol 2010;48:1078-84.
49. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002;10(3).
50. Mohammad I, Zafar I, Javid H, Hidayat H, Manzoor A, Asma E, Muhammad I.C. Chemical constituents and antioxidant activity of Geranium Wallichianum. Rec. Nat Prod 2009; 3: 193-7.
51. Bondet V, Brand-Williams W, Berset C. Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. Foof Sci Technol 1997; 30: 609-15.
52. Jain PK, Agrawal RK. Antioxidant and free radical scavenging properties of developed mono-and polyherbal formulations. Asian J Exp Sci 2008;22:213-20.
53. Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Bahramian F, Bekhradnia AR. Antioxidant and free radical scavenging activity of H. officinalis L. var. angustifolius, V. odorata, B. hyrcana and C. speciosum. Pak J Pharm Sci 2010;23:29-34.
54. Lahvale, Manish S., Mishra S.H. Evaluation of free radical scavenging activity of Butea monosperma Lam. Indian J Exp Biol 2007;45: 376- 84.

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