



## EVALUATION OF ETHANOLIC POLYHERBAL FORMULATION FOR ANTI OXIDANT ACTIVITY

V. Satyanarayana\*, S. Jaya Kumari

Research Scholar, School of Pharmaceutical Sciences, Vel's University, Chennai-117, Tamil Nadu.

\*Corresponding author E-mail: [veeragandamsatya@gmail.com](mailto:veeragandamsatya@gmail.com).

### ARTICLE INFO

#### Key Words

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### ABSTRACT

**Aim:** The present work Carried out the anti oxidant activity of different polyherbal formulations containg different portion of ethanolic extract. Materials& Methods Based on litreture surveyi have selected traditional used Four *plants Polygonum glabrum, Canthiumdicoccum, Ochnaobtusata, Argyreia nervosa* 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. **Results and Discussion:** 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 the two formulations F2 having the high flavonoid content and this can be used for futher evaltulion for pharmacological activities.

### 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 rigorous 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 an antioxidant compound. Amongst the most important exogenous antioxidants, vitamin E, vitamin C,  $\beta$ -carotene, vitamin E, flavonoids, mineral Se are well known, but also vitamin D and

vitamin K<sub>3</sub>. 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].

**Polygonumglabrum** which is commonly called as dense flower knotweed is a semi aquatic perennial plant. It belongs to the family polygonaceae and genus polygonum<sup>[19]</sup>. 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<sup>[20]</sup>. In some areas the root stock is used for the treatment of jaundice and piles<sup>[21]</sup>. The leaves are used as an antimalarial agent in sudan<sup>[22]</sup>. In south India Polygonumglabrum leaves are used for the treatment of dysentery<sup>[23]</sup>. A decoction of the leaves and seeds are used as cardiogenic, astringent and anthelmintic<sup>[24]</sup>. The whole plant decoction is used as a remedy for colic pain, pneumonia and the boiled paste is applied in cuts and wounds<sup>[25]</sup>. Apart from medicinal use, the whole plant is powdered and used as bait for fishing. Peels from stem are used for treating rheumatism<sup>[26]</sup>.

**Ochnaobtusata DC.**(FamilyOchnaceae).  
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 Ochnaobtusata is used for ulcer, asthma and bronchitis<sup>[27-29]</sup>. From the source of literature documentation and relevant traditional approaches on plant drugs,

**Canthiumdicoccum** also known as nallabalu (telugu), nallamandharam (tamil) in India belongs to the family Rubiaceae. The plant is found in deccan peninsula, maharashtra southwards, and extending from bihar eastwards to assam and Meghalaya. Malakafe is an unarmed, 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-β-D-glucopyranosyl)-rutin<sup>[30]</sup>. Diglycosides, rutin and its benzoic derivative, 7-O-(5-O-benzoyl-β-D-glucopyranosyl)-rutin from C dicoccum and kaempferol 3-β-D-rutinoside from C rheedii strongly inhibited all test fungi.<sup>[31]</sup> Ethanolic extract of whole plant of Canthiumdicoccum 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.<sup>[32,33]</sup> ethanolic extract of Canthiumdicoccum for anti-diabetic in an alloxan induced diabetic rat model. Results 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.<sup>[34]</sup> Ethanolic extract for anti-arthritic activity in albino rats. Results showed significant anti-arthritic activity against Egg-albumin induced arthritis model.<sup>[35]</sup> 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 of the constituents provide scientific bases and evidence for antimicrobial, anti-tumor, immunomodulatory, and antioxidant properties of the plant.<sup>[36]</sup>

*Argyrea nervosa* has been used widely by the tribals of Rajasthan to prevent conception while in Assam and Bihar leaves are used as vegetable. In Uttar Pradesh folklore practice the young leaves are used for healing the wound<sup>[37]</sup> Young leaves are used to treat wounds and skin infections. According to Yuani medicine, roots are used in rheumatic affections, alternative tonic to cerebral disorders and as diuretic<sup>[38]</sup>. Roots preparations are used to treat syphilis, synovitis, rheumatism, obesity, wound, ulcers, skin infections. Roots are used as an appetitizer, aphrodisiac, brain tonic, cardiogenic, expectorant, and in anti-inflammatory. In Hindu medicine, root is used externally for to reduce obesity<sup>[39,40]</sup> Among the phytoconstituents ergoline alkaloids are the major constituents in *Argyrea nervosa*. It is one of the essential phytoconstituents of *Argyrea nervosa* from the historical and quantitative point of view. A group of nineteen indole alkaloids were identified and isolated by thin layer chromatography and paper chromatography. Among those constituents ergoline alkaloids, lysergic acid and isolysergic acid were analysed by thin layer chromatography, m.p, ultraviolet and infrared spectral analysis. Seeds are found to possess hypotensive and spasmolytic activity which were due to the mixture of ergot alkaloids, isolated and analysed by

ultraviolet. Due to instability only one constituent was identified as ergometrine. Other constituents such as caffeic acid and ethyl caffeate were identified<sup>[41,42]</sup> Apart from ergoline alkaloids, N- formyllooline alkaloids, flavonoid sulphates, steroids and triterpenoids were isolated from other parts of *Argyrea nervosa*<sup>[43,44]</sup> Para-hydroxycinnamate, scopelitin and argyroside<sup>[45,46]</sup> isolated oil from the seed of *Argyrea nervosa* and evaluated the antibacterial effect<sup>[47]</sup> The plant contains ergoline alkaloids showed hallucinogenic effect and used for recreation purposes

Materials and method:

### Plant source and authentication

*Polygonum glabrum*, *Ochna obtusata* DC, *Canthium indicum* and *Argyrea nervosa* 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.<sup>[48]</sup> 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 Specimen Voucher no:1972,1220,1012,2162 Preserved for further reference at our laboratory.

### Chemicals

Freund's adjuvant complete (CFA), N-methoxysuccinyl-Ala-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-nitrobenzoic acid (DTNB), Nitroblue tetrazolium (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 *Polygonumglabrum*, *Canthiumdicoccum*, *Ochnaobtusata*, *Argyreianervosa* were collected and dried. Then the material was blended to form a fine powder and extracted with Ethanol using Soxhlet apparatus for 6 hrs at 50°C 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 <sup>[48]</sup>

DPPH radical scavenging activity The DPPH assay method is based on the reduction of DPPH, a stable free radical. 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 DPPH-H 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:

$$\% \text{ scavenging} = \left[ \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \right] \times 100$$

#### Nitric oxide free radical scavenging activity <sup>[52, 53, 54]</sup>

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 nitroprusside 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. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions.

**Table no1: Different types of formulations using Ethanolic extracts of four different plants**

S. no	Different ratios of ethanolic extract of four plants			
	<i>Polygonumglabrum</i>	<i>Canthiumdicoccum</i>	<i>Ochnaobtusata</i>	<i>Argyreia nervosa</i>
Formulation I	1	1	1	1
Formulation II	2	1	1	1
Formulation III	1	2	1	1
Formulation IV	1	1	2	1
Formulation V	1	1	1	2

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

**Table no2: DPPH Radical Scavenging Assay for crude Ethanolic extract of five different poly herbal formulations.**

S.No	Concentration(µg/ml)	%Anti-oxidant activity				
		F1	F2	F3	F4	F5
1	10	8.4	10.6	10.8	10.6	10.6
2	20	16.6	23.6	22.6	23.6	23.6
3	40	35.8	43.8	43.6	43.8	43.8
4	60	56.6	61.2	61.2	61.6	62.2
5	80	76.6	82.3	81.4	81.8	79.4
6	100	89.8	98.6	98.2	99.6	94.8
7	IC50	54.5	56.9	48.6	52.08	49.08

**Table no3: Nitric oxide free radical scavenging activity for crude Ethanolic extract of five different poly herbal formulations.**

S.no	Concentration(µg/ml)	%Anti-oxidant activity				
		F1	F2	F3	F4	F5
1	10	9.4	9.6	10.8	8.6	10.6
2	20	19.6	23.6	22.6	19.21	21.6
3	40	39.8	41.8	43.6	41.8	41.8
4	60	61.2	62.2	59.2	57.4	59.2
5	80	78.2	82.3	79.4	73.2	72.4
6	100	92.4	96.6	92.2	82.6	84.8
7	IC50	51.5	58.8	50.2	54.1	53.6

**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
5	F5	8.96±0.554	0.812

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 IC<sub>50</sub> value for each test compounds as well as standard preparation were calculated

$$\% \text{ scavenging} = \left[ \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \right] \times 100$$

#### Estimation of total flavonoid content <sup>[50]</sup>

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 % AlCl<sub>3</sub>, 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.

#### Estimation of total phenol content <sup>[49]</sup>

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.

#### Discussion

The present experimental procedure used for evaluation of in vitro anti oxidant activity of different formulations (F1,F2,F3.F4,F5 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 five formulations formulation F2 and F4 having the higher. Ic 50 values respectively 58.8 and 54.1 and The Total Phenolic and flavonoid content is rich in the F2 formulation when compared with standard.

#### Conclusion

From this result Poly herbal formulation F2 having *Polygonumglabrum*, *Canthiumdicocccum*, *Ochnaobtusata*and, *Argyrea nervosa* respectively in the ratio of 2, 1, 1, and 1 is having the better anti oxidant activity and flavonoid content. hence this Polyherbal formulation of ethanolic extract can be used for further studies to know the specific pharmacological activities.

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