The present work carried out the antioxidant activity of different polyherbal formulations containing different portion of ethanolic extract. Based on literature 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 further evaluation for pharmacological activities.

Key words: polyherbal formulation, antioxidant activity, Polygonum glabrum, Canthium dicoccum, Ochna obtusata, DPPH, NO-scavenging, flavonoid.
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 of traditional properties. The tribes of chattisgarh use the root stock is used for the treatment of jaundice and piles [21]. In south india, Polygonum glabrum leaves are used for the treatment of dysentery [22]. In south India, Polygonum glabrum leaves are 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, maharashtra 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 diarrhoea it contains a new flavonol glycoside, 7-O-(5-O-benzoyl-β-D-glucopyranosyl)-rutin [30]. Diglycosides, rutin and its benzoic derivative, 7-O-(5-O-benzoyl-β-D-glucopyranosyl)-rutin from C dicoccum and kaempferol 3-B-D-rutinoside from C rheedii strongly inhibited all test fungi. [31] 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 [32, 33].The ethanolic extract of Canthium dicoccum for anti-diabetic in an alloxaan 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. MATERIALS 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
(1, 1-Diphenyl –2-picrylhydrazyl) was dissolved in 3.3 ml antioxidant (H-A) was shown in figure 2. 4.3 mg of DPPH present. The scavenging reaction between (DPPH.) and an violet colour (although there would be expected to be 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 radical DPPH-H form results in decolorization (yellow colour) with less 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:

\[
\% \text{ scavenging} = \frac{[\text{Absorbance of control} - \text{Absorbance of test sample}]}{\text{Absorbance of control}} \times 100 \ldots \text{(Equation 1)}
\]

### 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 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.

\[
\% \text{ scavenging} = \frac{[\text{Absorbance of control} - \text{Absorbance of test sample}]}{\text{Absorbance of control}} \times 100 \ldots \text{(Equation-2)}
\]

### 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 | Ochna obtusata |
| 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| 96.8| 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 evaluation 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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