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PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTI BACTERIAL ACTIVITY OF *SENECIO TENUIFOLIUS BURM* FAMILY: *ASTERACEAE*

*S.APARNA[1], K.N.V RAO[1], ASIA BEGAM[1], DAVID BANJI[1]

Nalanda College of Pharmacy, Cherla Pally, Nalgonda

*Corresponding Author Email: aparna_sama@yahoo.com

ABSTRACT

Senecio is a very large genus of flowering plants in the sunflower family (Asteraceae). Senecio tenuifolius is distributed all over the Indian peninsula. This plant is regarded as a medicinal plant is commonly found in the dry hills of southern India that extends from southern Maharashtra to Kerala and east wards through Karnataka and Tamilnadu. The aerial parts of the plant were taken and subjected to extraction with alcohol. The extracts was screened for phytochemical screening, total phenolic content, total flavonoid content and Anti bacterial activity against the Gram-positive (Streptococcus aureus, Bacillus subtilis) and Gram-negative (Escherischia coli, Klebsiella pneumonia) organisms using cup-well method. From experimentation, the extracts were found to contain alkaloids, flavonoids, steroids, glycosides, carbohydrates and phenolic compounds in various concentrations. Anti bacterial studies revealed that strain specific concentration is screened with the extracts and zone of inhibition is compared with that of concentrations and standard. Also control vehicle (alcohol) is maintained. Gentamycin, the standard antibacterial drug used was effective in inhibiting these bacteria. The inhibition increased in the dose dependent manner from 50mg, 100mg, and 150mg. Zone of inhibition has been shown only for the Bacillus subtilis and E.Coli.

KEY WORDS

Senecio tenuifolius, Asteraceae, Anti bacterial activity, Gentamycin.

INTRODUCTION

The Indian medicinal plant, *Senecio tenuifolius* is slender and glabrous much branched annual herb. The *Senecio tenuifolius* grows at an altitude of 50cm tall. The flowering occurs between September and December. The propagation of plant by using seeds [1].

Leaves are sessile, pinnatifid / bipinnatifid. All the segments of *Senecio* are slender, spreading and obtuse in shape. They are available with a few scattered bracts. These are 10 to 13 involucral bracts which are oblanceo-late. Leaf size is 3-4mm long, acute/ acuminate. These bracts are available with broad margins and yellowish nerves and 6 to 10 ligues. *Senecio* is green colour plant height is 15 to 45cm. Achenes of Ray flowers are elongated , and there is pappus copious and yellowish in color, in addition to that are as long as the achenes. And in terminal heads, heterogamous. Fruits are Achene, obovate, ribbed. Pyrrolizidine alkaoids have been found in *Senecio* sp[2]. And other are senkirkine, O-acetyl senkirkine and integerrimine were isolated from stems and leaves [3, 4]. Resistance to Anti bacterial agents is a major global public health problem. Infectious diseases account for approximately one-half of all death in tropics. Despite the progress made in the understanding of microorganisms and their control in industrialized nations, incidents due to

drug resistant microorganisms and the emergence of unknown disease causing microbes, posed enormous public health concern [5].

Staphylococcus aureus, Escherischia coli, Bacillus subtilis and Klebsiella pneumoniae are now resistant to virtually all of the older antibiotics [6]. This resistance is largely due to indiscriminate use of Anti bacterial drugs commonly used on the treatment of these infectious diseases. Furthermore some antibiotics have serious undesirable side effect which limit their application, so there is serious need to develop new Anti bacterial agents that are very effective with minimal unwanted side effect and higher plants represent a potential source of novel antibiotic prototype [7].

MATERIALS AND METHODS

a. Collection of plant:

Plant was collected in the forest regions of thalakona (Nelakona) regions of chitoor distict, by Dr. K. Madhava Chetty, Asst. Professor, Deparment of Botany, S.V. University, Tirupathi[8].

b. Extraction method

The aerial parts of plant were dried in shade and powdered and sieved with sieve no: 40 and extracted with alcohol. The extracts were distilled and residual mass was air-dried till we get powdery mass. This is subjected to preliminary phytochemical screening, total phenolic content, total flavonoid content and Anti bacterial activity.

c. Preliminary phytochemical screening: [9,10]

The successive extract of aerial parts of *Senecio* tenuifolus burm. were subjected to preliminary phytochemical analysis for various phytochemical constituents as per given standard procedures.

d. Estimation of total phenolic content:

The total phenolic content of successive extracts of aerial parts of *Senecio tenuifolus burm* was determined according to the Folin–Ciocalteau method [11,12], with slight modifications. Series of gallic acid standard solutions at a concentration range of 20-200 µg/ml and test sample 1000 µg/ml in methanol were prepared. 0.5ml of each gallic acid dilution was mixed with 5ml of 10% reagent and 4ml of 1M aqueous Sodium Carbonate. The mixture was allowed to stand for 15 min and absorbance of reaction mixture (blue colour) was measured at 765nm using UV visible spectrophotometer. Standard graph was plotted with the series of concentrations (20-200 µg/ml) on X-axis and absorbance on Y-axis. The total phenolic concentrations were determined from standard graph and results were expressed in terms of mg Gallic acid equivalents (GAE)/gm dry extract.

d. Estimation of total flavonoid content

Total flavonoid content of successive extracts of aerial parts of *Senecio tenuifolus* burm was determined by aluminium chloride colorimetric method [13]. Series of rutin standard solutions at a concentration range of 10-100 µg/ml in methanol and 1000 µg/ml of sample were prepared. 1ml of sample is mixed with 3ml of methanol, 0.2ml of 10% aluminium chloride, 0.2ml of 1M potassium acetate and 5.6ml of distilled water, and then allowed to stand at room temperature for 30min. The absorbance of the reaction mixture (pink color) is measured at 415nm. Standard graph was plotted with the series of concentrations (10-100µg/ml). flavonoids are expressed in terms of mg of rutin equivalents/gm dry extract.

e. Anti bacterial screening of extracts:

Anti bacterial assay of aerial parts of *Senecio tenuifolus burm* was determined using agar well diffusion method as adopted by Kumar et al. (2010) [14].

Test organisms: [15]

Purchased from IMTECH (MTCC Chandigarh)

- **Staphylococcus aureus -- MTCC 3160** } **Gram-positive**
- **Bacillus subtilis – MTCC 736**
- **Escherichia coli -- MTCC 739** } **Gram-negative**
- **Klebsiella pneumonia – MTCC 3384**

PROCEDURE

These bacterial strains were sub cultured in nutrient broth at 37˚C. Inoculums were added to the molten nutrient agar medium. Mixed well and poured in to the petriplates then left to solidify at room temperature. Using the sterile cork bore the wells (6 mm) were made in each petriplate. Various concentrations of extracts (50,100,150 mg/ml) were pour in to each wells with the help of micropipette. Standard antibiotic (Gentamycin 5µg/ml) was used and vehicle control (water/alcohol) is maintained and packed tightly with parafilm. Then the petriplates were incubated at 37˚C for study of their bacteriostatic and bacteriocidal action at 24 and 48 hours. After the incubation period, the diameter of the zone of inhibition of each well was measured and compared with that of standard and control.

RESULTS AND DISCUSSION

According to preliminary phytochemical screening, aerial parts of *Senecio tenuifolus Burm* found to contain alkaloids, flavonoids, triterpenoids, glycosides and carbohydrates which exhibit the class of pharmacological activities like Anti bacterial, Anti-oxidant activities.

Total phenolic content in alcoholic extract was found to be 66.18 µg/ml. Total flavonoid content in alcoholic extract was found to be 75.42µg/ml.

Anti bacterial activity showed the profound activity at 150mg/ml concentration when compared to standard and control.

Phenols are very important plant constituents with multiple biological functions including antioxidant activity because of their radical scavenging ability due to their OH groups. Many reports are available showing the relative correlation between phenol and antioxidant activity. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts [16].

Scavenging activity for free radicals of di (phenyl)-(2, 4, 6-trinitrophenyl) iminoazanium (DPPH) has been widely used to evaluate the antioxidant activity of natural products from plant and bacterial sources. The result of the present study showed that the in vitro alcoholic extract of Senecio *tenuifolus Burm*. Contained highest amount of phenolic compounds and exhibited the maximum antioxidant activity.

The high scavenging property of alcoholic extracts in in vitro *Senecio tenuifolus Burm*, may be due to hydroxyl groups existing in the phenolic compounds, chemical structure that can provide the necessary component as a radical scavenger [16]. The results obtained above are in consonance with other researches which have also shown a directly proportional relationship between Phenol and antioxidant activity [17].

The improved DPPH method used in this study to systematically assess the total antioxidant capacity of the medicinal plant extracts on a large scale, being simple, fast, reliable and inexpensive. This efficient and effective method can be used for systematic screening of

medicinal and dietary plants for their relative antioxidant content.

Several studies have revealed that intake of natural antioxidants is correlated with low incidence of cancer, heart diseases, diabetes, and other diseases associated with ageing [18, 19].

Determination of the natural antioxidant compounds of plant extracts will help to develop new drug candidates for antioxidant therapy [20, 21, 22,23].

The plants may be considered as good sources of natural antioxidants for medicinal uses such as against ageing and other diseases related to radical mechanisms [24, 25].

Morphological evaluation of successive extracts of *Senecio tenuifolus Burm*

Table 1: Colour, Consistency and yield of successive extracts of aerial parts of *Senecio tenuifolus Burm*

	Benzene	Chloroform	Ethyl acetate	Ethanol	Water
% Yield	1.2g	1g	1.45g	1.88g	1.5g
Consistency	Waxy	Oily	Oily	Viscous	Waxy
Colour(Daylight)	Greenish black	Green	Green	Red	Light green
Short UV	Black	Light yellow	Light black	Light brown	Black
Long UV	Green	Light black	Light green	Red wine	Light brown

Total phenolic content:

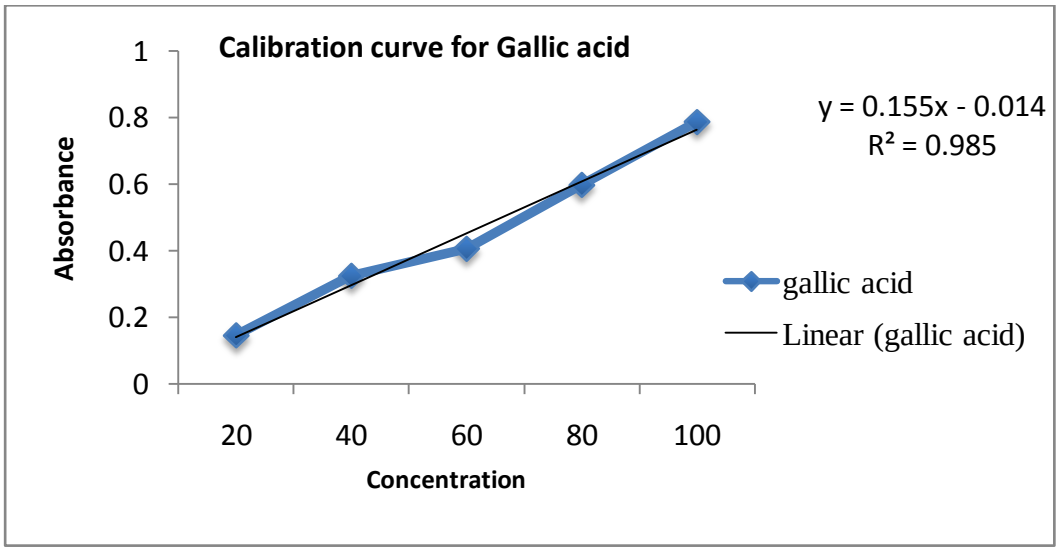

Figure: 1 Calibration curve for Gallic acid

Total flavonoid content:

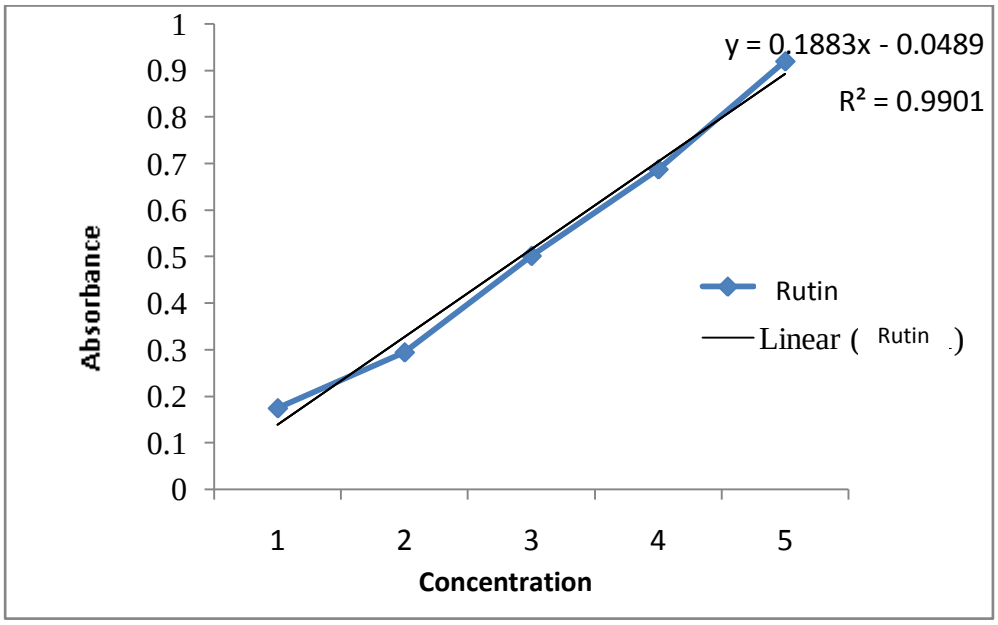

Figure: 2 Calibration curve for rutin

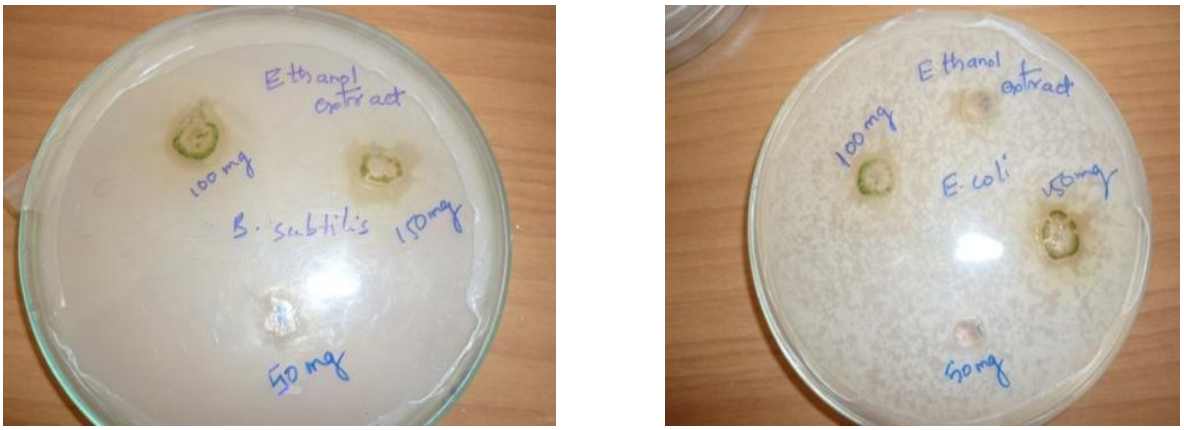

Figure: 3 Anti bacterial activity of alcohol extract of *Senecio tenuifolus Burm*

Table: 2 Anti bacterial activity of alcoholic extracts of *Senecio tenuifolus Burm* against various organisms with different concentrations (50,100,150mg/ml). Std-Gentamycin (5 µg/ml)

Microorganisms	50mg (alcoholic extract)	100mg (alcoholic extract)	150mg (alcoholic extract)	control	Standard 5µg/ml	Standard 10µg/ml
Escherechia coli	1mm	2mm	4mm	_	18mm	19mm
Bacillus subtilis	2mm	4mm	5mm	_	15mm	17mm
Staphylococcus aureus	_	_	_	_	14mm	15mm
K.pneumoniae	_	_	_	_	_	_

Values represent mean ± SD, n=3. Bore diameter-6mm.

CONCLUSION

From the above results, it is concluded that *Senecio tenuifolus Burm* found to contain considerable Anti bacterial activity in dose dependent manner. Effective concentration for activity was at 150mg/ml. There was a good correlation between % inhibition of DPPH by the plant extract and the total phenolic content, total flavonoid content. Natural antioxidants of plant origin have greater application and they can also be used as nutraceuticals and phytoceuticals as they have significant impact on the status of human health and disease prevention [26].

The inhibitory activities of the extracts live up to their potential in the treatment of bacterial induced ailments or diseased conditions, in line with the traditional use of plant extracts. This investigation thus provides a scientific basis for the use of the plant extracts in home-made remedies and their potential use in the treatment of bacterial-induced ailments.

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***Corresponding Author:**
****S.Aparna***
Nalanda College of Pharmacy Cherla Pally, Nalgonda
Email: aparna_sama@yahoo.com