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RESEARCH ARTICLE

TLC BASED PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF SENNA ALATA

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Abstract

Senna alata is an ornamental flowering plant also known as ringworm bush and candle bush belongs to Leguminaceae family and used mainly as antifungal agent. The plant is pharmacological significant and used as antibacterial, antioxidant, anti-cancerous, anti-mutagenic, antifungal, anti-diabetic and antiviral activities. Leaves of the plant have anti-parasitic activity used in treatment of eczema, ringworm, asthma, bronchitis and in poisonous insect bites, bark of the plant is used in treatment of skin diseases. In the present study chloroform, methanol, petroleum ether and aqueous extracts were used for phytochemical analysis, TLC profiling and antioxidant activity of Senna alata. The phytochemical analysis of Senna alata indicated presence of tannins, saponins, flavonoids, alkaloids, terpenoids, steroids, cardiac glycosides and quinones in Senna alata. In TLC analysis maximum 6 bands were found in leaf extract, 5 bands in stem extract and 4 bands were found in root methanol extract. The plant showed good antioxidant activity in methanol and aqueous based leaf, stem and root extract which may be due to the presence of flavonoids and phenolic compounds. The plant has potent antioxidant and antimicrobial properties so characterization of bioactive molecules of the plant should be beneficial for formulation of drugs.

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Introduction:-

Medicinal plants play key role in global economy as they are important for approximately 85% of traditional medicine preparations. Plants produce bioactive molecules which have various biological activities and used for treatment of several diseases. Senna alata (L) Roxb (Cassia alata L) is an important annual tropical plant belongs to Leguminosae family and widely distributed in India, Indonesia, Brazil, all over Africa and America including Nigeria (Adelowo and Oladeji, 2017). The plant has many vernacular names such as ringworm bush, candle bush, emperor candle stick, christmas candle, acapulo, and calabra bush. Anthraquinones and polyphenols are the main bioactive molecules and other compound includes tannis, saponins, alkaloids, steroids, flavonoids and carbohydrates. Leaves of Senna alata contain anthraquinones such as emodin, aloe-emodin, and rhein and are the main constituents of the plant and possess laxative activity. Several secondary metabolites are present in the plant such as emodin (Prasenjit et al., 2016), quercetin, 5,7,4'-trihydroflavanone, kaempferol-3-O- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside, fatty acids, chrysoeriol, kaempferol-3-O- β -D-glucopyranoside, hydrotetra-triacontane,

n-dotriacontanol, kaempferol, n-triacontanol (Liu et al., 2009), aloe-emodin-O- β -glucoside, kaempferol, aloe-emodin, rhein methyl ester (Duong et al., 2013), 2, 5, 7, 4' tetrahydroxy Isoflavone and 5, 7-dihydroxy-3', 4'-dimethoxy flavones (Rahman et al., 2015), kaempferol (Rhazri et al., 2015), and volatile oil (Igwe and Onwu, 2015). Presence of secondary metabolites in the plant exhibits various pharmacological properties such as antifungal, anti-tumor, antioxidant, antibacterial, anti-inflammatory, analgesic, and immune stimulating activities have been reported from its leaf, stem, flower, root-bark and seed extracts. Isah et al. reported that *Senna alata* is used for curing of skin infections, diabetes mellitus, sickle cell anemia and malaria. Abubakar et al. reported ellagitannin, n-hexadecanoic acid, naphthalene, phenolic acids, purine and xanthone compounds in *Senna alata*. Traditionally leaf part of the plant is used in treatment of skin diseases such as ringworm, itching, scabies, eczema, ulcers and other related disease (Igoli et al. 2005). Whole parts of the plant have medicinal values such as leaves were used for skin infection, seeds as anti-helminthic, roots for uterus disorder (Herman et al. 1978). Esimone reported that extracts of plant are used for cosmetic formulation in several skin care products and dehydrated leaves were used in herbal formulation such as herbal soaps, shampoos, tincture and herbal tea. Bejoy et al. reported that the extracts of plants are used as anti-inflammatory, diuretic and CNS depressants. The plant is known for antifungal, antidiabetic, anticancer, anti-inflammatory, anti-genotoxic, hepatoprotective, hypolipidemic, antioxidant, antimicrobial and anti-estrogenic effects¹⁴. Flavonoids and phenol play important role in antioxidant activity of plants by absorbing and neutralizing free radicals (Sarkar et al. 2014). Rutin and quercetin is a natural flavones derivative having strong free radical scavenging property used in treatment of several diseases. The pharmacological properties of rutin include antibacterial, antiviral, antitumour, anti-protozoal, anti-allergic, anti-inflammatory, hypolipidaemic and vasoactive (Calabro, 2005). Gallic acid is obtained from tannic acid by hydrolysis with H_2SO_4 and the biological properties of gallic acid includes radical scavenging, anti-fungal, anti-cancer, anti-inflammatory, and chemoprotective (Soong and Barlow, 2006). Abo et al. reported that *Senna alata* was used in treatment of skin disorders such as ringworm, hernia, blennorrhagia, diabetes, constipation, hemorrhoids, intestinal parasitosis and syphilis. The different biological properties exhibited by the compound give motivation for isolation of bioactive molecules from natural sources. Oxidative stress arises due to imbalance between formation of reactive oxygen species and antioxidant defense system which may damage DNA, carbohydrate, proteins, and lipids (Halliwell and Gutteridge, 1992). Antioxidant is a defense mechanism that prevents the oxidation of other substances in low concentration the reactive oxygen species include hydroxyl radical, hydrogen peroxide, nitric oxide superoxide anion and per oxy-nitrite anion (Nagendrappa, 2005). The aim of the present study was to evaluate the phytochemical, TLC analysis and antioxidant potential of the plant in chloroform, methanol, petroleum ether and aqueous extracts.

Materials and Methods:-

Collection and identification of plants:

The leaf, stem and root parts of the plant were collected from Durg district, Chhattisgarh, India and authenticated by Botanical Survey of India, Allahabad. The parts of the plant were washed; shade dried and grinded to make powder. Powdered samples were used for preparation of extracts in soxhlet apparatus.

Preparations of Extracts:

10gms of each powder was taken in conical flask and 100ml of each solvent (methanol, chloroform, petroleum ether, aqueous) were added separately. Soxhlet apparatus is used for preparation of extracts with different solvents of their increasing polarity. Extraction were done until the solvent in soxhlet became colorless then each extract was collected in separate bottles. The extracts were stored at $-20^{\circ}C$ and used for phytochemical analysis.

Qualitative phytochemical analysis:

Phytochemical screening was carried out by following the standard protocol of Brindha et al. and Edeoga et al. for the presence of alkaloids, glycoside, steroids, tannins, flavonoids, saponin quinones, terpenoids and gum.

TLC analyses:

Thin layer chromatography is used for separation of bioactive molecules of the plant. Silica gel 'G' is used for preparation of TLC plates, homogeneous suspension of silica gel was sprayed over the plates and dried in air. TLC plates were activated in hot air oven at $110^{\circ}C$ for 30 min and after cooling the plates were used for chromatography. Methanol based leaf extracts were used as sample and the mobile phase contain ethyl acetate: methanol: water in ratio (36:36:28). The plates were visualized under UV- Transilluminator for detection of spots and the R_f values were calculated by using following formula

$$R_f \text{ value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

DPPH-Method:

DPPH-free radical scavenging activity of methanol, chloroform, petroleum ether and aqueous extract of *Senna alata* were examined by following the standard protocol (Koleva et al., 2002). DPPH in its radical form absorbs at 517 nm but its absorption decreases due to reduction by an antioxidant or a free radical species. DPPH solution (0.3 mM) was prepared in methanol and kept in dark bottle to protect it from light. Control solution was prepared by dissolving 1ml of DPPH in 3ml of methanol solution. Test sample contains varying concentration (0.2, 0.4, 0.6, 0.8, 1 mg/ml) of different extracts and 1ml of DPPH was added and final volume of 4ml is made up with methanol. The tubes were incubated in dark for 30 minutes and reading was taken at absorbance 517nm in UV-Spectrophotometer. Ascorbic acid was taken as standard using methanol as diluents. The percentage inhibition by sample treatment is determined by the formula:

$$\% \text{ of inhibition of DPPH activity} = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{\text{Absorbance of control}} \times 100$$

Results and Discussion:-**Phytochemical analyses:**

Secondary metabolites are very important compounds present in plant extracts as they play various biological activities and contribute in traditional medicine. Phytochemical analysis is very important as it provides knowledge about the nature of bioactive compounds present in plant extract. *Senna alata* is pharmacologically significant plant used in treatment of several diseases such as skin related diseases, diabetes, malaria, wound healing etc. Senthil kumar et al. reported presence of terpenoid, cardiac glycosides and steroids whereas absence of alkaloids in leaf, flower and seed coat extract of *Senna alata*. Uwangbaoje reported absence of steroid in leaf extract. It has been reported that in leaf extract of *Senna alata* presence of flavonoid and glycosides was detected (Moriyama et al., 2003). Previously several authors have reported phytochemical analysis in leaf, flower, seed and stem part. In the present study we have evaluated phytochemical and antioxidant activity of leaf, stem and root part in petroleum ether, chloroform, methanol and aqueous extract. Results of phytochemical analysis as shown in (Table 1, 2 and 3) showed presence of tannin, saponin, alkaloid, flavonoid, cardiac glycoside, steroids, terpenoids, quinones whereas gum was found absent in all parts and extracts of the plant. In leaf extract of *Senna alata* glycoside and alkaloid was found in petroleum ether, tannin, flavonoid, glycoside, quinones and steroid was found in chloroform extract. In methanol extract all the phytochemicals were present except gum whereas in aqueous extract saponin, tannin, flavonoids and quinones were found. In stem extracts cardiac glycoside, alkaloids and quinones were found present in petroleum ether and chloroform extract whereas tannin, terpenoid, flavonoid, glycoside and quinones were found in methanol extract whereas saponin, tannins and quinones were found in aqueous extract. In root extract saponin and alkaloid were found in petroleum ether extract, tannin glycoside, alkaloid and quinones were found in chloroform extract, saponin, tannin, steroid, glycoside and quinones were found in methanol extract. Tannin and quinone were found in aqueous root extract. Saponins are bitter, insoluble in water and exhibit blood coagulating activity form foam in aqueous solution and possess hemolytic activity. Ramadas et al. reported that flavonoids are water soluble having antioxidant and anticancer activity. Flavonoids known as “nature’s biological response modifiers” protect plant from pathogen attack. Flavonoids are polyphenolic compounds exhibit anti-ulcer, anti-hepatotoxic, anti-allergic, anti-viral, anti-cancer, anti-inflammatory activities. They are potent antioxidants capable of scavenging ROS due to presence of phenolic hydroxyl group (Cao et al., 1997). Alkaloids, tannins and flavonoids showed medicinal activity against pathogens and are used in treatment of various diseases (Usman and Osuji, 2007). Tannin and flavonoids are responsible for anti-diarrheal activity (Enzo, 2007). Essiett and Bassey reported presence of saponins, terpenoids, anthraquinone, tannins, alkaloids, and a trace amount of steroids in *Senna obtusifolia*. The phytochemicals of the plant exhibit various biological activities and may be used for pharmaceutical importance.

Table 1:- Showing phytochemical screening of leaf extracts of *Senna alata*.

Phyto compounds	Petroleum ether	Chloroform	Methanol	Aqueous
Saponin			+	+
Tannin		+	+	+++
Terpenoids			+	
Flavonoids		+	++	+
Steroids		+	+	
Cardiac glycoside	+	+	+	—
Alkaloids	+	—	+	—
Quinones	—	+	++	+

Gum				
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Table 2:- Showing phytochemical screening of stem extracts of *Senna alata*.

Phytocompounds	Petroleum ether	Chloroform	Methanol	Aqueous
Saponin	+			+
Tannin			+	++
Terpenoids			+	
Flavonoids			+	
Steroids				
Cardiac glycoside	+	++	+	
Alkaloids	+	+		
Quinones	+	+	+++	+
Gum				

Table 3:- Showing phytochemical screening of root extracts of *Senna alata*.

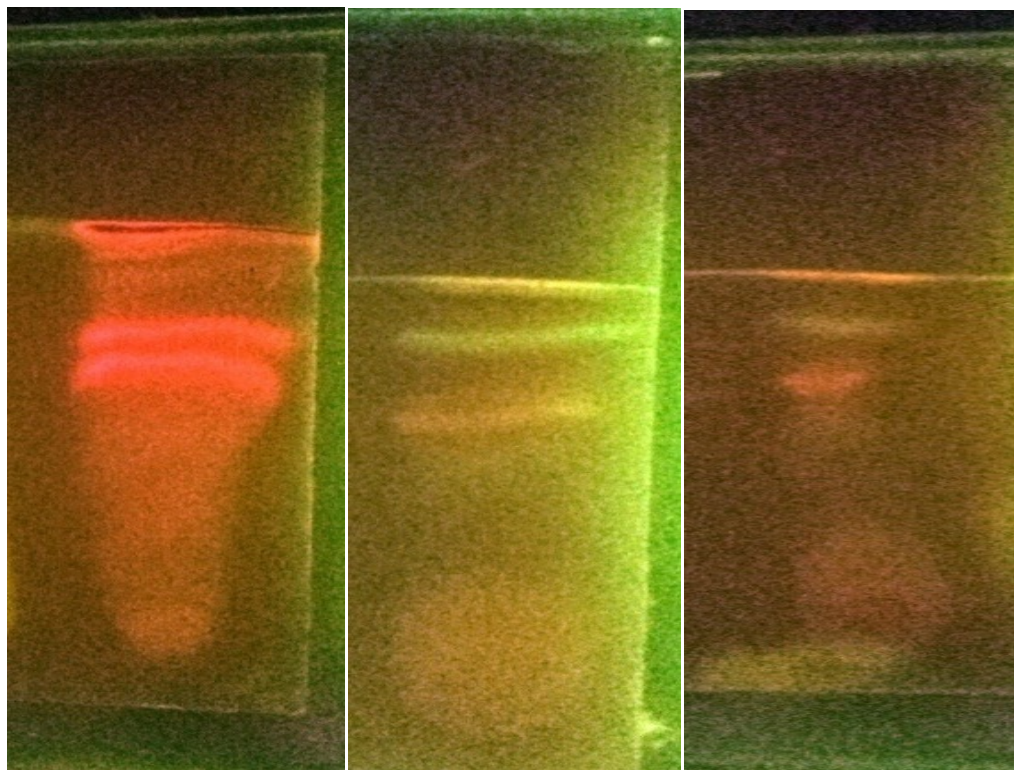
Phytocompounds	Petroleum ether	Chloroform	Methanol	Aqueous
Saponin	++		+	
Tannin		+	++	++
Terpenoids				
Flavonoids				
Steroids			+	
Cardiac glycoside		++	+	
Alkaloids	+	+		
Quinones		+	+++	+
Gum				

Thin layer chromatography:

TLC analysis was performed using ethyl acetate: methanol: water (36:36:28) as mobile phase (Table 3) in methanol based leaf, stem and root extracts of plant and compounds were identified by comparing R_f values of sample with standard compounds reported by Phansawana and Pongsabangphob and Ananthi and Keerthana. In leaf extract maximum 6 spots were found in which R_f value 015, 057, 080 and 083 showed presence of tannic acid, quercetin and rutin compound respectively. In stem extract maximum 5 spots were obtained among which 015, 024, 085 showed presence of tannic acid, gallic acid and rutin. In root extract 4 spots were found in which R_f value 011, 084 showed presence of tannin and rutin.

Table 4:- Showing TLC retention factor (R_f Value) for methanol extract of *Senna alata* and standard.

SNo	Parts	R_f values	Standard R_f values
1	Leaf	015,057,066,069,080,083	Gallic acid = 024, Rutin = 083 Tannic acid = 015 Quercetin = 057
2	Stem	015, 024,072,085,094	
3	Root	011,030,084,097	



I.

Fig 1:- Showing TLC profiling of leaf, stem and root methanol extract of *Senna alata*.

Antioxidant activity:

The antioxidant activity of leaf, stem and root extracts of plant was estimated by DPPH free radical scavenging activity. DPPH method is fast, easy and cheap method to measure the antioxidant activity in food samples (Kirtikar and Basu, 1975). Ascorbic acid is used as standard and absorbance was taken at 570nm. The optical density of the sample decreases due to decolorization of purple colour of DPPH to yellow colour which may be due to pairing of DPPH free radical with free radical species of sample (Ghosh, 1998). In leaf based extract of *Senna alata* the antioxidant activity was found in methanol, aqueous and petroleum ether extract and absent in chloroform extract. As, on increase the concentration of sample the optical density decreases due to increased percentage of scavenging of free radicals. The antioxidant activity of ascorbic acid was found highest. In stem extract antioxidant activity was found in methanol, aqueous and chloroform extract where as in root part methanol and aqueous based extracts. Methanol and aqueous extract showed potent antioxidant activity in leaf, stem and root part which may be due to presence of flavonoids and phenolic compounds. Panichayupakaranant et al. reported antioxidant activity in methanolic extracts of *Senna alata* by DPPH method. Pharmacological and clinical studies suggested that flavonoids exhibit radical scavenging activity against free radicals and acts effectively in inhibiting oxidation of lipoproteins and prevents various human diseases (Ames, 1993; Heinonen et al., 1998; Rice Evens, 1996). *Senna alata* showed significant scavenging effect on the DPPH free radical and the optical density of the sample decreases with increasing concentration of sample extract which may be due to presence of flavonoids and phenolic compound. The percentage of free radical scavenging activity was calculated by using formula and the radical scavenging activity increases with increasing sample extracts. Antioxidant activity is evaluated mostly by DPPH method due to difference in free radical scavenging activity of different compounds the rate of reaction of DPPH differs in different compounds (Janaszewska and Bartosz, 2002). Sagnia et al. reported antioxidant activity of *Senna alata* in ethanol extract against superoxide anion and hydrogen peroxide. Methanol extract of *Senna alata* showed strong antioxidant activity due to presence of phenols, flavonoids, carotenoids, anthraquinones, vitamin-c and vitamin-A (Chatterjee et al. 2013). Similarly in our present study also methanol and aqueous extract showed good antioxidant activity in all the parts of the plant which attribute to the presence of flavonoids, alkaloids, tannins, and phenolic compounds which was also detected in TLC analysis. Presence of anthroquinones, phenols, flavonoids in *Senna alata* indicates strong DPPH radical scavenging activity.

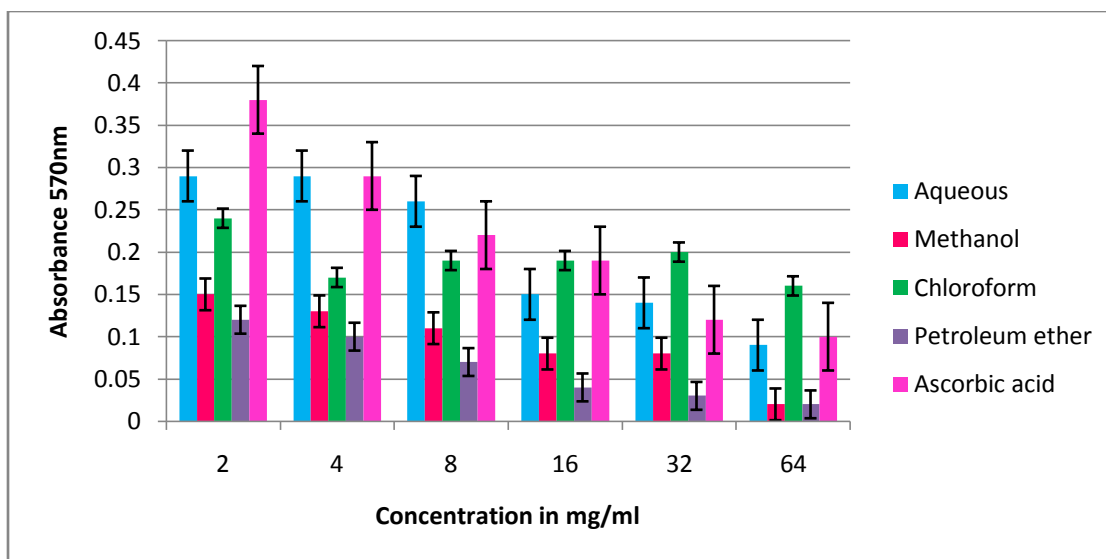


Fig 2:- Showing antioxidant activity of leaf extract of *Senna alata* by DPPH assay.

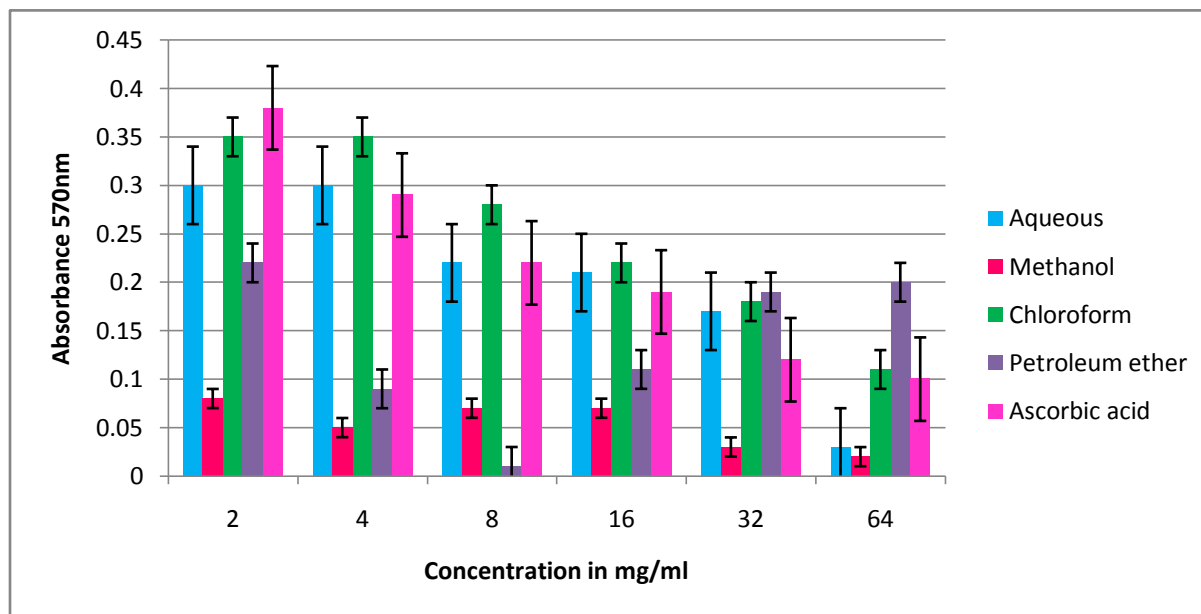


Fig 3:- Showing antioxidant activity of stem extract of *Senna alata* by DPPH assay.

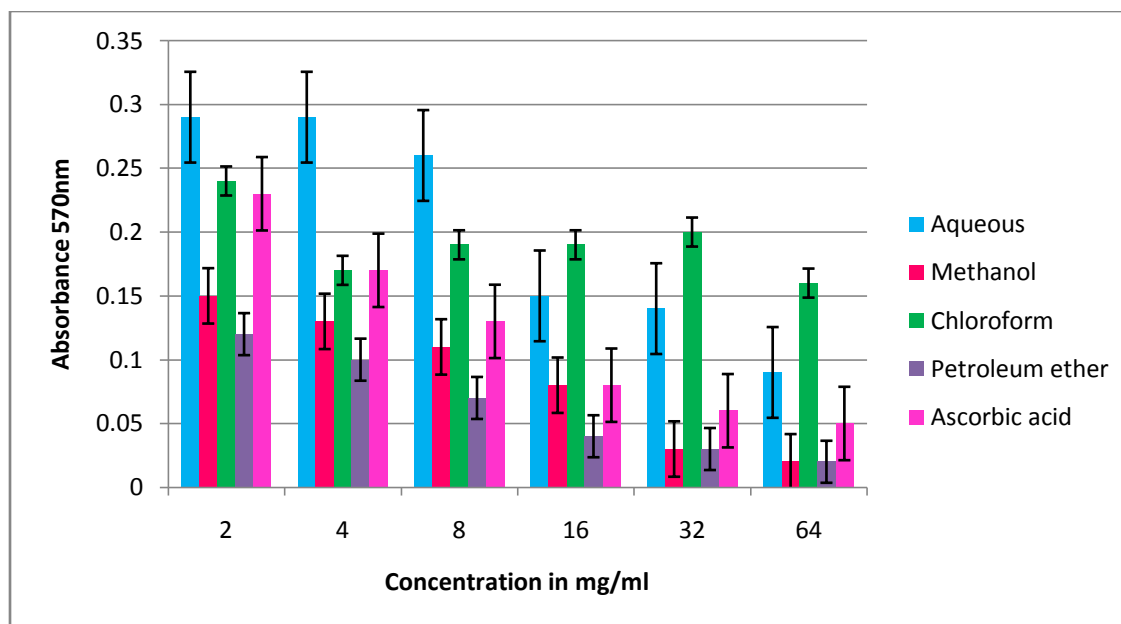


Fig 4:- Showing antioxidant activity of root extract of *Senna alata* by DPPH assay.

Conclusion:-

Senna alata is a traditional medicinal plant known for its ethno pharmacological features and valued for laxative effect and used in treatment of several skin diseases such as ringworm and scabies. Many authors have contributed for detection of bioactive molecules of this plant. In the present study we have detected phytochemicals in four extracts from leaf, stem and root part by phytochemical and TLC analysis. The secondary metabolites detected in plant include tannin, saponin, alkaloid, glycoside, quinones, flavonoid and steroids in different extracts. The plant showed potential antioxidant activity in methanol and aqueous based leaf, stem and root extracts. Our finding suggests the importance of plant used as a source of natural antioxidant to prevent oxidative stress and also used to inhibit antimicrobial growth. Thus this study should be beneficial for detection and structure elucidation for pharmaceutically lead compound.

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