IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 15, Issue 5 Ser. I (Sep. –Oct. 2020), PP 52-64
www.Iosrjournals.Org

In Vitro Antioxidant activity of leaves and stem bark of *Saraca indica* L.

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Abstract: *Saraca indica L. (Ashok) is a small evergreen tree of sub family Caesulpinoideae of family Leguminosae. The leaves are paripinnate, oblong and rigidly sub- coriaceus with 6- 7 leaflets. Bark of this tree is rich in tannins, flavonoids, steroids, volatile oil, glycosides, and various steroidal glycosides. Leaves contain various carbohydrates, tannins, gallic acid and egallic acid. Flowers are rich in sarcasin, sarcadin, waxy substances, proteins, carbohydrates and steroids. Seeds of this plant contain various fatty acids like oleic, linoleic, palmitic and stearic acid. The aim of the present study is to assay the antioxidant activity of the leaves and bark extracts of Saraca indica in vitro.*

In the present investigation the antioxidant activity of the leaves and bark extracts of Saraca indica in vitro has been assayed. The results revealed that methanol and hexane extracts of leaves and bark of S. indica caused bleaching of the purple colour of DPPH radical that caused the development of pale spots over a purple background. This indicated that the extracts contained some active phytochemicals exhibiting antioxidant activities. The DPPH scavenging activity of the methanol and n-hexane extracts (0.5 -2.5 mg/ml) exhibited concentration-dependent free radical scavenging activity. The extracts (0.5-2.5 mg/ml) and the standard antioxidant n-propyl gallate (3.0 mg/ml) caused a concentration- dependent reduction of Fe3+ to Fe2+. The extracts (0.5 – 2.5 mg/ml) and n-propyl gallate (3.0 mg/ml) caused a concentration- dependent inhibition of linoleic acid autoxidation.

On the basis of the present results it can be concluded that the methanole and n-hexane leaf and bark extracts of this plant possess significant antioxidant activity.

Keywords: *Saraca indica, Antioxidant, DPPH, Linoleic acid, Bark, Leaves*

Date of Submission: 29-08-2020 Date of Acceptance: 14-09-2020

I. Introduction

Saraca indica L. (Ashok) is a small evergreen tree of sub family Caesulpinoideae of family Leguminosae. The leaves are paripinnate, oblong and and rigidly sub- coriaceus with 6- 7 leaflets (Ali, 2008) [1]. This tree has orange coloured flowers with a beautiful aroma, 7- 8 stamens are found in flower and fruits are smooth, leathery and flat pods including 6- 8 seeds inside (Jain, 1968) [2].Bark of this tree is rich in tannins, flavonoids, steroids, volatile oil, glycosides, and various steroidal glycosides. Leaves contain various carbohydrates, tannins, gallic acid and egallic acid. Flowers are rich in sarcasin, sarcadin, waxy substances, proteins, carbohydrates and steroids. Seeds of this plant contain various fatty acids like oleic, linoleic, palmitic and stearic acid.

Ashok tree has been an integral part of Indian history. It is commonly called a tree which is important to decrease our sorrows. It has got religious significance and is also worshipped by some people in parts of India. . It has a number of medicinal properties hence used by physicians since centuries in Unani system of medicine along with Ayurveda (Kokate *et al*., 2007) [3]. It is primarily used for the management of female reproductive problems. Married women in India are known to eat Ashoka flower buds as a ritual to invoke deities for child protection as well as gynecological problems. Women suffering from menorrhagia drink a decoction on an empty stomach in the morning, which is prepared from the bark of Ashoka in water in combination with other herbs such as *Terminalia chebula* and *Coriandrum sativum* (Begum *et al*., 2014) [4].. In leucorrhoea, the decoction of Ashoka bark in water and milk after evaporation of water is consumed by women. In India, Srilanka, Bangladesh and Pakistan Ashoka bark is used by womenfolk in treating menorrhagia, menstrual and uterine disorders (Mishra *et al*., 2013; Mollik *et al*., 2010) [5, 6]. Ashoka is blood purifier and

used in all skin diseases, ammenorhea, dysmenorrhea menopause, menorrhagia, painful menstruation blood circulation and purification, cancer, diarrhea, dysentery, edema, heart disease, hepatitis, herpes, jaundice, joint pain, kidney and gall stones, , paralysis, skin problems, rheumatoid arthritis, obstructions in urinary passages. Ashok is also a cardiac tonic that can act as a supportive therapy for people suffering from hypertension, circulatory problems, edema, congestive heart failure etc. Its bark has natural detoxification properties which make it very useful to improve skin complexion and keep the body free from toxins inside out. Its natural cleansing properties can help the body stay toxin free. When the body has a lot of toxic load free radicals are produced. These free radicals then start damaging the body cells and all signs of ageing, disease and malfunctions are produced.

Ashok bark acts as a coolant and helps to relieve thirst, excessive burning sensation, anger, emaciation, sweating etc. These are all common signs of pitta aggravation which can be relieved with the use of Ashok bark in different ways. It also has some digestive properties. Common problems of digestion like bloating, flatulence, burping, colicky pain in abdomen; ascites etc. can be relieved with the use of Ashok. It is not exactly a direct indication of the herb but it does help because all diseases have root from a malfunctioning gut and digestive system overtime.

Saraca indica is a rain- forest tree. It is native of Asia and South America. It is originally distributed in the central areas of Deccan plateau. It is also found in Western Ghats of the Indian subcontinent. It is also widely distributed in the center and the Eastern Himalayas and in the hills of Khasi, Garo and available in West Bengal. It is common to all parts of Indian and other countries. In India it is easily available in West Bengal, Kerala, Maharashtra, Andhra Pradesh and Meghalaya (Kokate *et al*., 2007; Prajapati *et al*., 2003) [3, 7].

Saraca asoca is reported to contain glycoside, flavonoids, tannins and saponins (P. Pradhan *et al*. 2010) [8]. The *asoca* tree has many health benefits and has long been used in traditional Indian medicine as a key ingredient in various therapies and cures. It is used as protective drug for spasmogenic, oxytocic, uterotonic, anti-bacterial, antiimplantation, anti-tumour, antiprogestational, antiestrogenic activity against menorrhagia and anti-cancer. One of the uses of the *asoca* herb is in the treatment of menstrual disorders associated with excessive bleeding, congestion, pain, dysmenorrhoea, abdominal pain, uterine spasms and miscarriage [16, 17, 18] (Pradhan *et al*., 2010; Mollik *et al*., 2010; Begum *et al*., 2014) [8, 9, 10] . It also has a nourishing effect on the circulatory system, thereby making it an effective remedy in arrhythmia and cardiac weakness (Swamy *et al*., 2013) [11]. The asoca herb also helps in encouraging urine flow and thus helps in treating conditions that cause painful urination. It also has specific analgesic properties and it is said to improve the complexion of skin (Mishra *et al*., 2013) [12]. The various phytoconstituents have been reported in leaves and bark of the plant. All parts of plant viz. bark, leaves, flowers are regarded as medicinally important and used as therapeutic agent in treatment of diabetes, cancer and hemorrhagic dysentery, bleeding piles, uterine infections and bacillary dysentery. An antioxidant molecule, the gallic acid has been reported in *Saraca asoca* flower (Singh, S *et al*., 2015) [13]. Dried flower buds are reported to have antibacterial activity (Pradhan *et al*., 2009) [14]. Aqueous suspension of *Saraca asoca* flower has antiulcer activity in albino rats (Maruthappan *et al*., 2010) [15]. *Saraca asoca* bark and flowers exhibit antitumour activity against DLA, S-180 and Ehrlich ascites carcinoma tumour cell lines (Cibin and Devi, 2012) [16]. Larvicidal activity has also been recorded (Mathew *et al*., 2009) [17]. Chemopreventive activity of flavonoid fraction of *Saraca asoca* is reported in skin carcinogenesis (Cabin *et al*., 2010) [18]. Flower extract is bitter in taste and bark has a stimulatory effect on endometrium and ovarian tissue and used in uterine fibrosis, menorrhagia, bleeding hemorrhoids and also as astringent.

Oxidation process is one of the important routes for production of free radicals and these high energy molecules may abruptly interfere with the normal metabolic activities of the body causing immense damage to the normal tissues (Sies, 1997)[19]. There is a close relationship between diabetes and oxidative stress and it has been observed that the free radicals are produced in the form of ROS (reactive oxygen species) which cause mitochondrial DNA mutation thus resulting in hypoglycemic memory (davy *et al*., 1999) [20]. Free radicals generated during diabetes interfere with vital organ tissues and may lead to cardiovascular complications, diabetic nephropathy, diabetic retinopathy, erectile dysfunction and diabetic neuropathy (Ann and David, 2000) [21]. Several plants are known for their efficacy to overcome these complications by enhancing the *in vivo* anti oxidant defense and provide protection against oxidative tissue damage (Roja *et al*., 2005) [22]. The SOD (Superoxide dismutase), CAT (Catalase), Vitamin E and C are some of the antioxidants which provide protection to the diabetic tissues (Durdi *et al*., 2005) [23] and their level of defense can be assessed by measuring the MDA concentration which is the end product of lipid peroxidation (Hostetter *et al*., 1981) [6].

Metabolic processes in the body generate highly reactive species, known as free radicals, which injure cellular molecules. Free radicals are highly reactive atomic or molecular species that contain an unpaired electron (Halliwell and Gutteridge, 1992) [25] which contributes to their high reactivity. Free radicals react quickly with the nearest stable molecule to capture the electron they need to gain stability. The "injured" molecule loses its electron, becoming a free radical itself. They can damage vital cellular components like nucleic acids, cell membranes and mitochondria, resulting in subsequent cell death. As all aerobic organisms

utilize oxygen during cellular respiration and normal metabolism, the generation of free radicals by biochemical cellular reactions and from the mitochondrial electron transport chain is inevitable (Buonocore and Groenendaal, 2007) [26]. The free radicals include reactive oxygen and nitrogen species such as superoxide ($O2.^-$), hydroxyl (OH.)-, peroxyl (ROO-), peroxinitrite ($\cdot ONOO^-$) and nitric oxide ($NO\cdot$) radicals. All these are produced through oxidative processes within the mammalian body (Abdel-Hameed, 2009) [27]. They may also be generated through environmental pollutants such as cigarette smoke, automobile exhaust fumes, radiation, air pollution and pesticides (Aquil *et al*., 2006; Tivwari, 2001) [28, 29]. To protect the cells and organ systems of the body against reactive oxygen and nitrogen species, humans have evolved a highly sophisticated and complex antioxidant protection system, that functions interactively and synergistically to neutralize free radicals. These antioxidants are capable of stabilizing or deactivating, free radicals before they attack cells. Antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase destroy toxic peroxides. In addition to antioxidant enzymes, non-enzymatic molecules play important roles in antioxidant defence systems. These non- enzymatic molecules are of an exogenous nature and are obtained from foods. They include *α*-tocopherol, *β*-carotene, and ascorbic acid, and such micronutrient elements as zinc and selenium (Aqil *et al*., 2006) [28]. Normally, there is a balance between free radical generation and scavenging (Beris, 1991) [30]. Oxidative stress results from an imbalance between excessive generation of oxidant compounds and insufficient anti-oxidant defence mechanisms (Sies, 1997) [19]. When the natural antioxidant mammalian mechanism becomes inadequate, the excess of free radicals can damage both the structure and function of cell membranes in a chain reaction leading to degenerative diseases and conditions such as Alzheimer‟s disease, cataracts, acute liver toxicity, arteriosclerosis, nephritis, diabetes mellitus, rheumatism and DNA damage which can lead to carcinogenesis (Abdel-Hameed, 2009) [27].

All cells in eukaryotic organisms contain powerful antioxidant enzymes. Endogenous antioxidants made in the body are believed to be more potent in preventing free radical damage than exogenous antioxidants. The major classes of endogenous antioxidant enzymes are the superoxide dismutases, catalases and glutathione peroxidases (Sies, 1997) [19], *α*-lipoic acid and coenzyme Q10. In addition, there are numerous specialized antioxidant enzymes reacting with and, in general, detoxifying oxidant compounds.

Superoxide dismutases are present in almost all aerobic cells and in extracellular fluids (Johnson and Giulivi, 2005) [31]. Superoxide dismutase enzymes contain metal ion cofactors that, depending on the isozyme, can be copper, zinc, manganese or iron. They catalyse the breakdown of the superoxide anion into oxygen and hydrogen peroxide (Zelko *et al*., 2002) [32]. Catalases, on the other hand, are enzymes that catalyse the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor (Chelikani *et al*., 2004) [33].

The aim of the present study is to assay the antioxidant activity of the leaves and bark extracts of *Saraca indica in vitro.*

II. Materials and Methods

Preparation of methanol and hexane extract: The stem barks and leaves of *Saraca indica* were collected from the campus of Gaya College, Gaya and the samples were washed with water and air-dried at room temperature for 7 days, then oven-dried at 40ºC to remove the residual moisture. The dried stem barks and leaves were pulverized and stored in air-tight container for future use. Methanol (polar solvent) and n-hexane (non polar solvent) were used as solvent for in vitro antioxidant assay. Equivalent amount of powdered samples of stem barks and leaves were extracted with methanol and n-hexane at room temperature for 3 days. Extraction was done in water bath at 60ºC. About 1 gm of the dried sample was added into the test tubes containing 5 ml of solvent (methanol and n-hexane) separately and was extracted at room temperature. The sample was homogenized with extraction buffer. The supernatant was collected after three rounds of extraction. The solvent was evaporated under reduced pressure in a rotary evaporator at 40^0C. To this thick paste colloidal silicon dioxide was added and dried in vacuum tube dryer. The methanol and n-hexane extracts of leaves and bark thus obtained were then stored separately in deep freezer at -20^0C until further test.

Antioxidant assay:

***In vitro* qualitative DPPH test:** The qualitative test for antioxidant activity was performed using the rapid DPPH radical scavenging assay (Cuendet *et al.,* 1997) [34]. 10 µl of the leaf and bark extracts were applied on silica gel plates 60 F254 (Merck, 0.25 mm thick) and allowed to dry completely. The plate was then sprayed with a solution of 2% DPPH in methanol. A pale yellow to white spot over a purple background indicated a radical scavenging activity of the particular extract.

Quantitative antioxidant assays of extracts

Reducing power assay: Reducing activity of the methane and n-hexane extracts was assayed as follows. Different concentrations (0.5 – 2.5 mg/ml) of both the extracts as well as the standard drug *n*-propyl gallate (3.0

µg/ml) were prepared in aqueous methanol (50% v/v) and n-hexane (50%v/v) separately and 1 ml each was taken into test tubes in triplicates. To the test tubes, 2.5 ml of sodium phosphate buffer (pH=7) and 2.5 ml of 1% potassium ferric cyanide solution was added. The contents were mixed well and incubated at 50^0C for 20 minutes. After incubation, 2.5 ml of 10% trichloroacetic acid was added and the mixture centrifuged at 3000 revolutions per minute for 10 minutes. After centrifugation 2.5 ml of supernatant was added to 2.5 ml of distilled water. To this about 1 ml of 0.1% ferric chloride was added. The absorbance was then recorded at 700 nm. A graph of absorbance was then plotted against the concentration of the extracts. Increase in absorbance was indicative of higher reducing power of the extract.

Scavenging of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical assay: The free radical scavenging activity of methanol and n-hexane extract was determined as follows. 1 ml each of the extracts (0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml in methanol and n-hexane) was added to 3 ml methanolic solution of DPPH solution (20 mg/l) in a test tube. The reaction mixture was kept at 25°C for 30 mins. The absorbance of the residual DPPH was determined at 517 nm in a spectrophotometer (Cecil CE 7200 spectrophotometer, Cecil instrument limited, Milton Technical Centre, England). One milliliter (1 ml) methanol (50%) and nehexane (50%) was added separately to 3 ml DPPH solution, incubated at 25 °C for 30 minutes and used as control. *n*-propyll gallate (3.75-30 µg/l) was used as a standard free radical scavenger. The absorbance decreases with increasing free radical scavenging ability. Results were expressed as percentages of blank (100%). The concentration required to cause a 50% decrease in the absorbance was calculated (EC50). Each test was carried out using three replicates. The % DPPH scavenging effect (% of control) of the antioxidant was calculated as follows:

$$\% \text{ DPPH scavenging effects} = (Ac - At)/Ac \times 100$$

Where

Ac = Absorbance of the control; At = Absorbance of the test drug/ extracts

Total antioxidant capacity assay: The assay is based on the reduction of molybdenum, Mo +6 to Mo +5, by the extracts and subsequent formation of a green phosphate-molybdate (Mo +5) complex at acidic pH [26]. Test tubes containing l ml each of the extracts 0.5-2.5 mg/ml) and 3 ml of the reagent solution (0.6 M sulphuric acid, 28 mM disodium phosphate and 4 mM ammonium molybdate) were incubated at 95^0C for 90 minutes. After the mixture had cooled to room temperature, the absorbance of the solution was measured at 695 nm. Four concentrations of ascorbic acid (0.025, 0.05, 0.1 and 0.2 mg/ml) was used to construct a calibration curve. A blank solution was prepared by adding every other solution but without extract or standard drug. The antioxidant capacity was expressed as mg of ascorbic acid equivalent (AAE) per g of extract. This procedure was used for both methanol and n-hexane extracts.

Determination of total phenolic content: The presence of phenol in the methanol and n-hexane extracts of *Saraca indica* was determined qualitatively using ferric chloride test. An intense positive colouration indicating the presence of phenols led to further quantification of total soluble phenols in the extract. The total phenol in the extract was determined by spectrophotometric assay using the Folin-Ciocalteu‟s reagent as described by Singleton *et al.,* (1999) [35] using tannic acid as standard.

1ml of the extracts (0.25-2 mg/ml) in distilled water was added to 1 ml Folin-Ciocalteu‟s reagent in a test tube. The content of the test tube was mixed and allowed to stand for five minutes at 25^0 C in an incubator. 1 ml of sodium bicarbonate solution (2%) was added to the mixture. The reaction mixture was allowed to stand for 2 hours with shaking at 25^0C in an incubator. The mixture was then centrifuged at 3000 rpm for 10 minutes and absorbance of the supernatant determined at 760 nm. Three replicates were prepared for each concentration of tannic acid and extracts. 1 ml distilled water was added to 1 ml Folin-Ciocalteu‟s reagent processed in the same way as the test drugs and used as blank. Tannic acid was used as reference. Four concentrations of tannic acid (0.025, 0.05, 0.1, 0.2 mg/ml) were used to construct a calibration curve and the total phenols expressed as mg of tannic acid equivalents (TAE)/g of extract.

This method depends on the reduction of Folin-Ciocalteau reagent by phenols to a mixture of blue oxides which have a maximal absorption in the region of 760 nm. The reaction equation is as follows:

Folin: Mo^{+6} (yellow) + è (from antioxidant) → Mo^{+5} (blue)

Where the oxidizing reagent is a molybdophosphotungstic heteropolyacid comprised of $3H_2O{\cdot}P_2O_5{\cdot}13WO_3{\cdot}5MoO_3{\cdot}10H_2O$, in which the hypothesized active centre is Mo^{+6}.

Linoleic acid auto-oxidation assay: The extracts (0.5-2.5 mg/ml) in absolute alcohol were compared with *n*-propyl gallate (3.0 µgm/l) in absolute alcohol as a reference antioxidant. 2 ml of the extract, 2 ml of 2.5% linoleic acid in absolute ethanol, 4 ml of 0.05 M phosphate buffer (pH =7) and 1.9 ml of distilled water were put into test tubes with a screw cap and placed in an oven at 40^0C in the dark for 7 days. After the seven day period, 2 ml each of the extracts and standard drug was added to 20 % aqueous trichloroactetic acid solution and 1 ml of 0.6 % aqueous thiobarbituric acid solution. This mixture was placed in boiling water bath for 10 minutes and

after cooling, was centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured 535 nm. Each test was carried out in three replicates. Percentage inhibition of lipid peroxidation by the test drugs was assessed by comparing the absorbance of the drug test with that of the control (linoleic acid mixture without any drug). Data was presented as percentage inhibition of lipid peroxidation against concentration. The % inhibition of linoleic acid autoxidation was calculated as follows:

$$\% \text{ inhibition} = 1 - \left(\frac{D - D_O}{C_O - C} \right)$$

Where
Co= (Full reaction mixture) is the degree of lipid peroxidation in the absence of antioxidant
C= is the underlying lipid peroxidation before the initiation of lipid peroxidation
D= is any absorbance produced by the extract/ linoleic acid mixture
Do= is the absorbance produced by the extract alone

Statistical analysis: All the experiments were conducted in replicates of three and data was recorded as mean value ± SE. The statistical analysis was performed by one way analysis of variance (ANOVA) and means were compared by least significance difference test ($P< 0.05$) using the SPSS statistical software package (SPSS, ver. 10.0; Chicago. IL, USA).

III. Results

Qualitative DPPH (2, 2-diphenyl-1-picrylhydrazyl) test for Antioxidant Activity: The methanol and n-hexane extracts of leaves and bark of *Saraca indica* caused bleaching of the purple colour of DPPH radical that caused the development of pale spots over a purple background. This indicated that the extracts contained some active phytochemicals exhibiting antioxidant activities.

Quantitative antioxidant assay of methanol and n-hexane extracts: The methods used to determine quantitative antioxidant activity of the methanol and n-hexane extracts of leaves and bark of *Saraca indica* included total phenolic content, total anti-oxidant capacity, reducing power, DPPH radical scavenging activity and linoleic acid autoxidation assays.

1. The total phenolic content of the methanol and n-hexane extracts was determined using the Folin- Ciocalteu's reagent and tannic acid was used as standard. The total phenolic content of the extracts was expressed as mg of tannic acid equivalents (TAE) per g of extract. The four different concentrations of each of the methanol and n-hexane extracts of leaves and bark were used for quantitative assay. The total phenolic content in methanol and n-hexane extracts of leaves and bark has been presented in Table-1and Fig-1.

Table-1: Total Phenolic content in leaf and bark extracts of *Saraca indica*

Concentration of methanol and n-hexane extract (mg/ml)	Leaf		Bark	
	Methanol extract	n-Hexane extract	Methanol extract	*n-Hexane extract*
	Mean mgTAE/g ±SE	Mean mgTAE/g ±SE	Mean mgTAE/g ±SE	Mean mgTAE/g ±SE
1.0	24.25 ±0.42	22.51 ±0.51	48.25±0.61	47.15±0.31
1.5	28.27 ±0.41	27.35 ±0.41	50.15±0.42	48.27±0.45
2.0	35.65±0.47	34.45±0.47	56.35±0.36	54.35±0.32
2.5	38.75±0.38	37.71±0.38	65.25±0.35	63.15±0.33

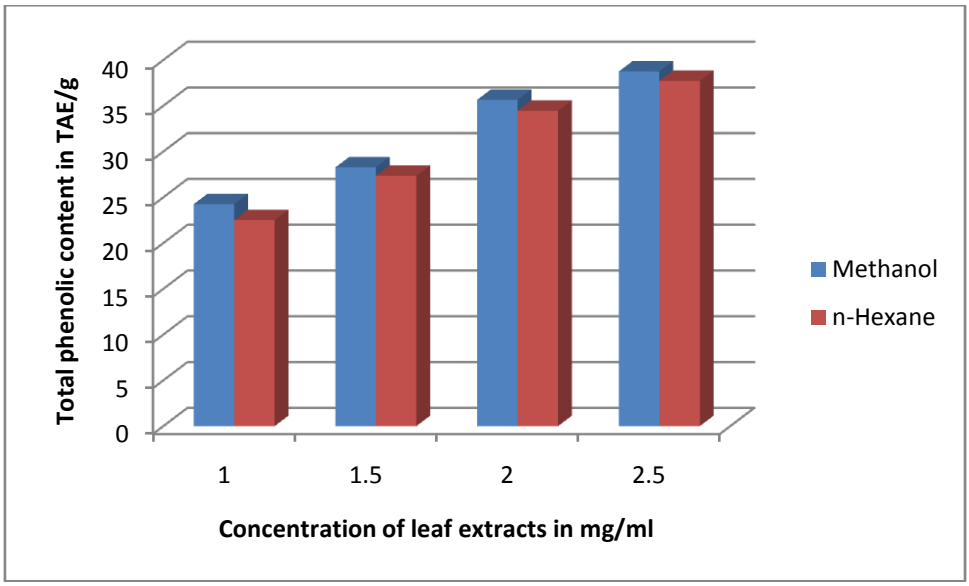

Fig-1: Total Phenolic content in leaf extracts of *Saraca indica*

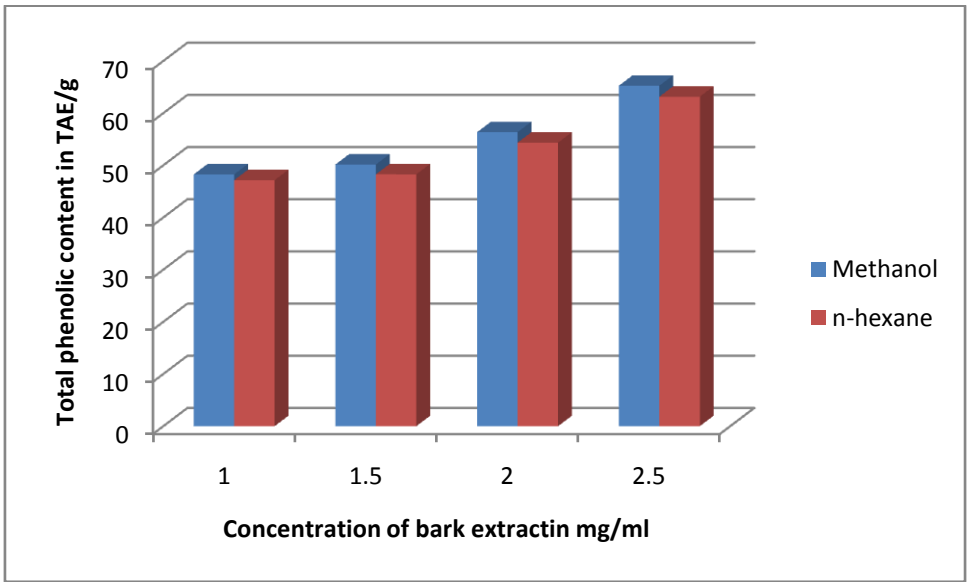

Fig-2: Total Phenolic content in bark extracts of *Saraca indica*

The results revealed that the leaves and bark of *Saraca indica* contained appropriate phenolics. The methanol extracts contained relatively high concentration of phenolics in comparison to n-hexane extracts. The total phenolic content in 1.0 mg/ml of methanol extract of leaves of S. *indica* was 24.25 ± 0.42 TAE/g. Their concentration increased on increasing the concentration of methanol leaf extracts. At 2.5 mg/ml of methanol leaf extract the concentration of total phenolics was 38.75 ± 0.38 TAE/g. In n-hexane extract of leaves of *S. indica* 1.0 mg/ml, 1.5 mg/ml, 2.0 mg/ml and 2.5 mg/ml of the extracts contained 22.51±0.51 TAE/g, 27.35 ± 0.41 TAE/g, 34.45 ± 0.47 TAE/g and 37.71 ± 0.38TAE/g phenolics respectively (Table-1; Fig-1). The bark extracts of *S. indica* contained more phenolics. In sample of methanol bark extracts 1.0 mg/ml, 1.5 mg/ml, 2.0 mg/ml and 2.5 mg/ml of extract contained 48.25 ± 0.61 TAE/g, 50.15 ± 0.42 TAE/g, 56.35 ± 0.36 TAE/g and 65.25 ± 0.35 TAE/g respectively, whereas the same concentration of n-hexane extracts contained 47.15 ± 0.31 TAE/g, 48.27 ± 0.45 TAE/g, 54.35 ± 0.32 TAE/g and 63.15 ± 0.33 TAE/g of phenolics respectively (Table-1; Fig-2). The results clearly indicated that the bark of *S. indica* contained more phenolic compounds in comparison to leaves.

2. Free Radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Scavenging Activity: The results of the free radical scavenging potential of methanol and n-hexane extracts of leaves and bark of Saraca indica using DPPH free radical scavenging method are depicted in Table-2 and Fig-3 and 4. The DPPH scavenging activity of the methanol and n-hexane extracts (0.5 -2.5 mg/ml) exhibited concentration-dependent free radical scavenging

activity (Table- 2; Fig-3 and 4). Low free radical DPPH scavenging activity was observed at 0.5 mg/ml of methanol and n-hexane extracts of both leaves and bark (IC_{50} 78.4 ± 0.024 µg/ml in methanol leaf extract, IC_{50}76.4 ± 0.021 µg/ml in n-hexane leaf extract, IC_{50} 79.4 ± 0.025 µg/ml in methanol bark extract IC_{50} 76.5 ± 0.026 µg/ml in n-hexane bark extract. The highest free radical scavenging activity was observed at concentration 2.5 mg/ml of both methanol and n-hexane extract (IC_{50} 925.5 ± 0.067 µg/ml in methanol leaf extract, IC_{50} 915.5 ± 0.085 µg/ml in n-hexane leaf extract, IC_{50} 922.7 ± 0.065 µg/ml in bark methanol extract and IC_{50} 916.5 ± 0.054µg/ml in bark n-hexane extract) (Table-2; Fig-3 and 4).

Table-2: DPPH scavenging activity of methanol and n-hexane extracts of leaves and bark of *Saraca indica*

Dose of extract in mg/ml	Methanol leaf extract	n-Hexane leaf extract	Methanol bark extract	n-Hexane bark extract
	IC50 (µg/ml) ±SEM		IC50 (µg/ml) ±SEM	
0.5	78.4 ±0.024	76.4 ±0.021	79.4 ±0.025	76.5 ±0.026
1.0	231.5 ±0.085	225.5 ±0.075	233.5 ±0.075	227.5 ±0.071
1.5	561.7 ±0.084	560.5 ±0.074	560.5 ±0.064	556.5 ±0.074
2.0	726.5 ±0.085	721.5 ±0.065	724.5 ±0.083	720.5 ±0.063
2.5	925.5 ±0.067	915.5 ±0.085	922.7 ±0.065	916.5 ±0.054

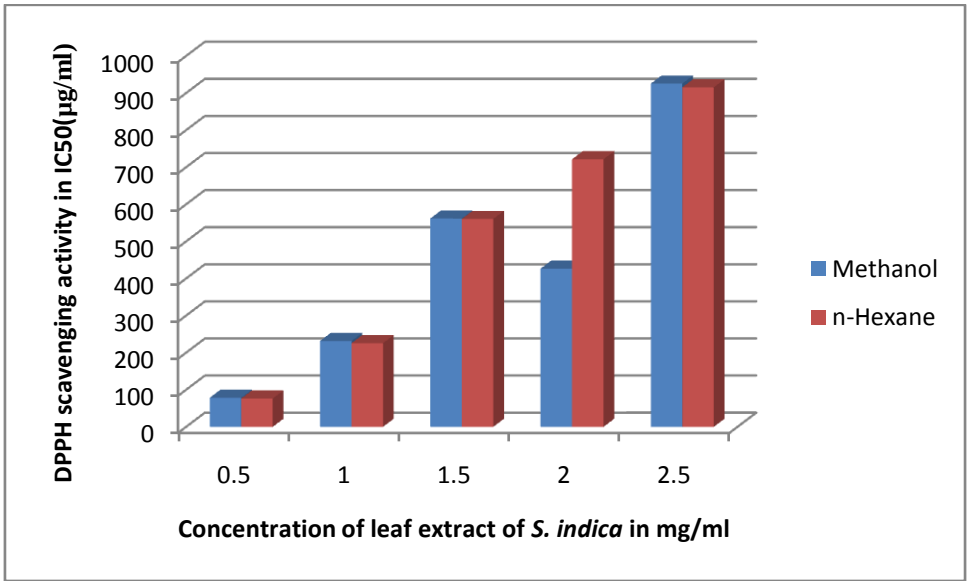

Fig-3: DPPH scavenging activity of methanol and n-hexane extracts of leaves of *Saraca indica*

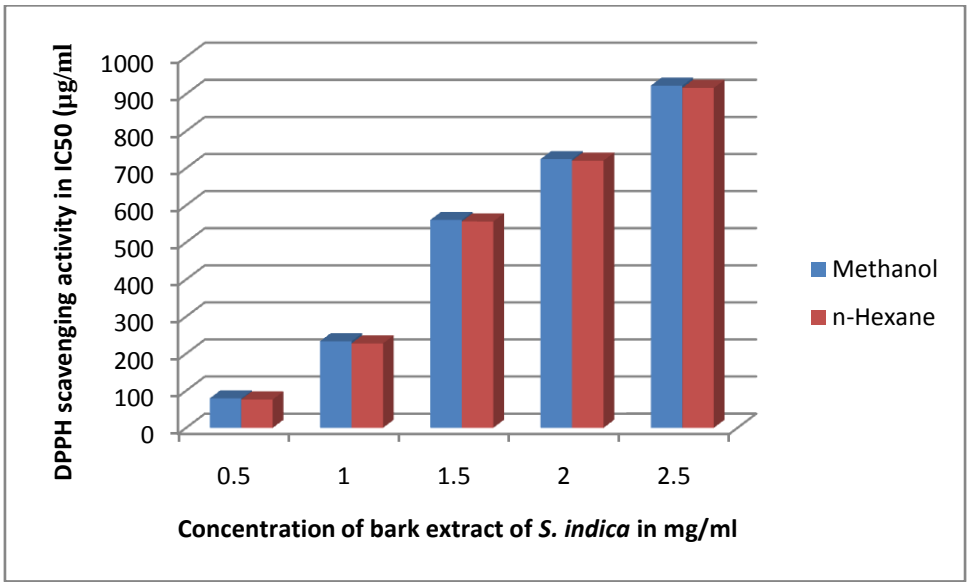

Fig-4: DPPH scavenging activity of methanol and n-hexane extracts of bark of *Saraca indica*

3. Reducing power: The extracts (0.5-2.5 mg/ml) and the standard antioxidant *n*-propyl gallate (3.0 mg/ml) caused a concentration – dependent reduction of Fe3+ to Fe2+. From the IC50 values (Table-3; Fig-5 and 6), the methanol and n-hexane extracts of leaves and bark of *S. indica* showed the highest reducing power. The reducing power activity of leaf extracts was relatively less in comparison to bark extracts. At concentrations of 0.5 mg/ml of methanol and n-hexane extracts of leaves the reducing power activity was IC_{50} 185.15 ± 0.014 µg/ml and IC_{50} 182.25 ± 0.013µg/ml respectively. In the same concentrations methanol and n-hexane extracts of bark caused slightly higher reducing power activity (IC_{50}189 ± 0.013 µg/ml for methanol extract and IC_{50} 186.16 ± 0.023 µg/ml for n-hexane extract). Maximum reducing power activity was noticed for both methanol and n-hexane extract from leaves and bark of *S. indica* at concentration of 2.5 mg/ml. At this concentration of extract the reducing power activity for methanol leaf extract, n-hexane leaf extract, methanol bark extract and n-hexane bark extract was IC_{50} 965.45 ± 0.035 µg/ml, IC_{50} 935.26 ± 0.015 µg/ml, IC_{50} 971.45 ± 0.031 µg/ml and IC_{50} 965.36 ± 0.027 µg/ml respectively (Table-3; Fig-5 and 6).

Table-3: Reducing power of methanol and n-hexane extracts of leaves and bark of *Saraca indica*

Dose of extract in mg/ml	*Methanol leaf extract*	*n-Hexane leaf extract*	*Methanol bark extract*	*n-Hexane bark extract*
	IC_{50} (µg/ml) ±SEM	IC_{50} (µg/ml) ±SEM	IC_{50} (µg/ml) ±SEM	IC_{50} (µg/ml) ±SEM
0.5	**185.15 ±0.014**	**182.25 ±0.013**	**189.15 ±0.013**	**186.16 ±0.023**
1.0	**225.35 ±0.016**	**212.17 ±0.016**	**235.17 ±0.012**	**231.12 ±0.016**
1.5	**562.25 ±0.026**	**560.45 ±0.011**	**571.25 ±0.015**	**563.15 ±0.017**
2.0	**725.15 ±0.029**	**716.25 ±0.016**	**755.15 ±0.017**	**746.25 ±0.025**
2.5	**965.45 ±0.035**	**935.26 ±0.015**	**971.45 ±0.031**	**965.35 ±0.027**
3.0 n-PG	**70.25 ±0.003**	**70.25 ±0.003**	**70.25 ±0.003**	**70.25 ±0.003**

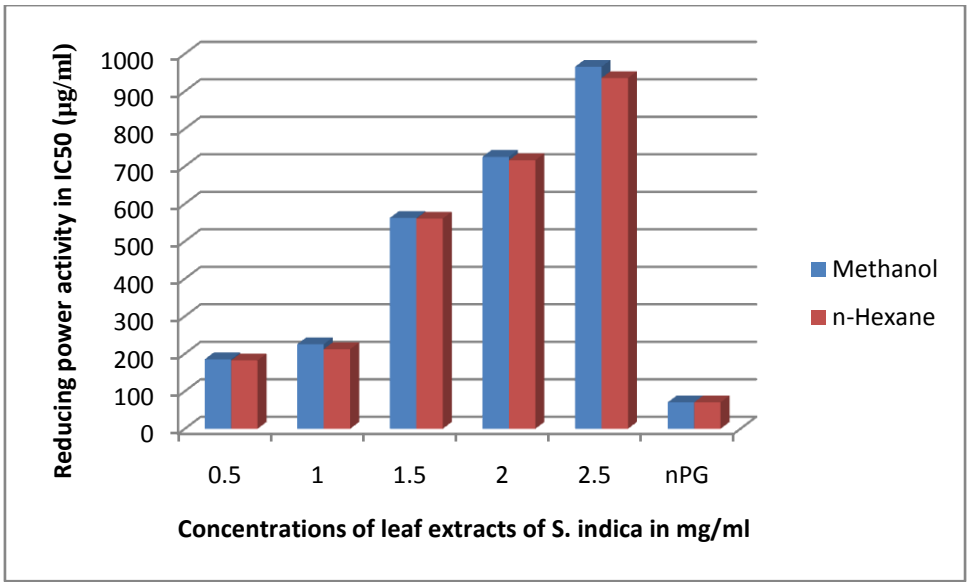

Fig-5: Reducing power activity of methanol and n-hexane extracts of leaves of *S. indica* in IC50 (µg/ml)

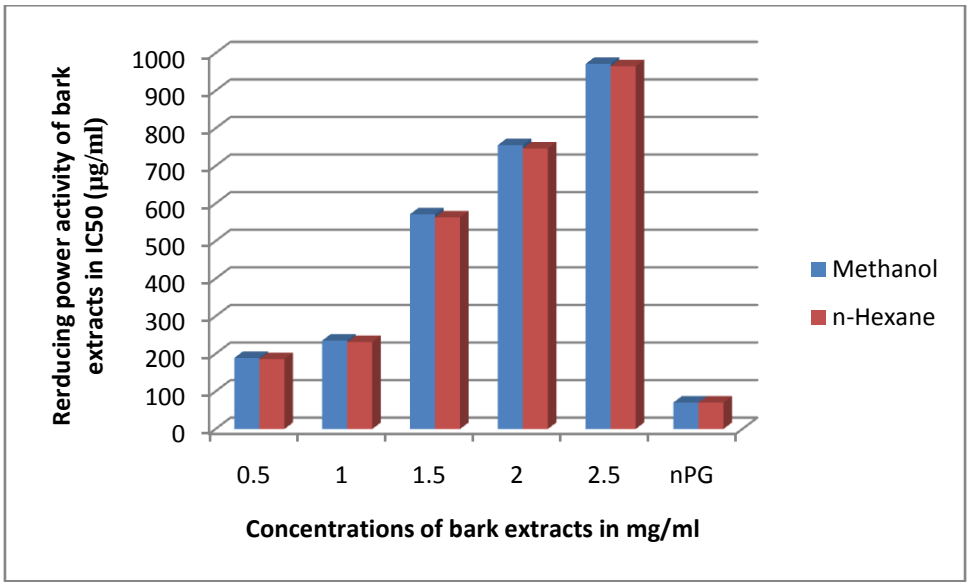

Fig-6: Reducing power activity of methanol and n-hexane extracts bark of S. *indica* in IC50 (µg/ml)

4. Lipid peroxidation: The ability of the methanol and n-hexane extracts and test drug to inhibit linoleic acid auto-oxidation was investigated. The extracts (0.5 – 2.5 mg/ml) and *n*-propyl gallate (3.0 mg/ml) caused a concentration- dependent inhibition of linoleic acid autoxidation (Table-4; Fig-7 and 8). The per cent inhibition of lipid peroxidation by methanol and n-hexane extracts of *S. indica* increased with increasing the concentration of methanol and n-hexane extracts of both leaves and bark. At concentration of 0.5 mg/ml of leaf and bark extracts caused minimum inhibition of lipid peroxidation (31.5 ± 0.15% by methanol leaf extract; 30.5 ± 0.13% by n-hexane leaf extract; 34.5 ±0.14% by methanol bark extract and 32.5 ±0.12% by n-hexane leaf extract). Percent inhibition of lipid peroxidation increased on increasing the concentration of leaf and bark extracts. At concentration of 2.5 mg/ml all the four extracts caused maximum inhibition of lipid peroxidation. At this concentration methanol leaf extract, n-hexane leaf extract, methanol bark extract and n-hexane bark extract caused 93.5 ± 0.21%, 90.7 ± 0.14%, 96.5 ± 0.20% and 92.5 ± 0.21% inhibition of lipid peroxidation respectively (Table-4; Fig-7 and 8).

Table-4: % inhibition of lipid peroxidation by methanol and n-hexane extracts of leaves and bark of *Saraca indica*

Dose of extract in mg/ml	*Methanol leaf extract*	*n-Hexane leaf extract*	*Methanol bark extract*	*n-Hexane bark extract*
	% inhibition of lipid peroxidation ±SEM	% inhibition of lipid peroxidation ±SEM	% inhibition of lipid peroxidation ±SEM	% inhibition of lipid peroxidation ±SEM
0.5	31.5±0.15	30.5±0.13	34.5±0.14	32.5±0.12
1.0	57.6 ±0.13	55.4 ±0.12	61.5 ±0.16	60.6 ±0.17
1.5	83.7±0.21	81.5±0.11	87.4±0.15	84.5±0.16
2.0	90.2 ±0.21	87.2 ±0.15	93.5 ±0.21	90.4 ±0.15
2.5	93.5 ±0.21	90.7 ±0.14	96.5 ±0.20	92.5 ±0.21
3.0 n-PG	65.5 ±0.12	65.5 ±0.12	65.5 ±0.12	65.5 ±0.12

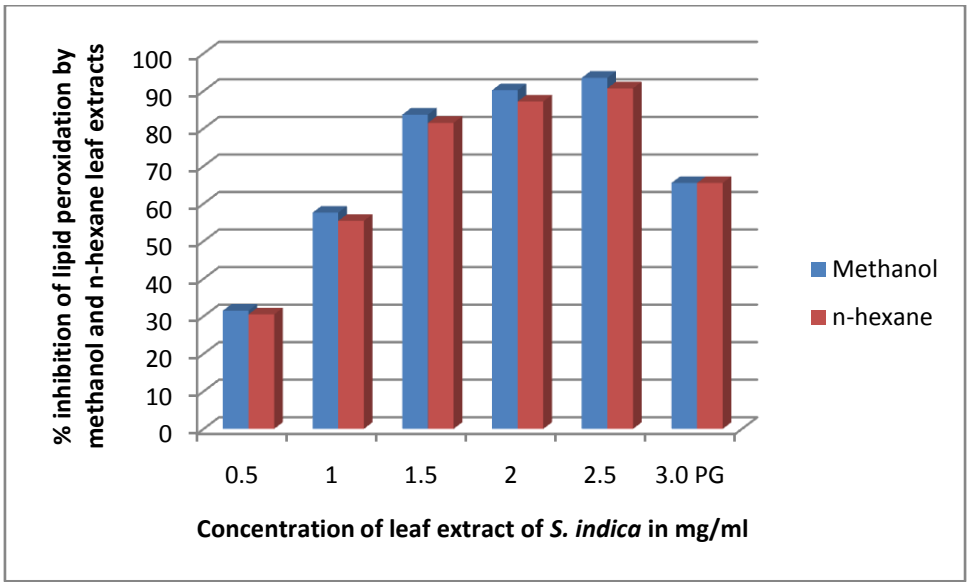

Table-7: % inhibition of lipid peroxidation by methanol and n-hexane extracts of leaves of *Saraca indica*

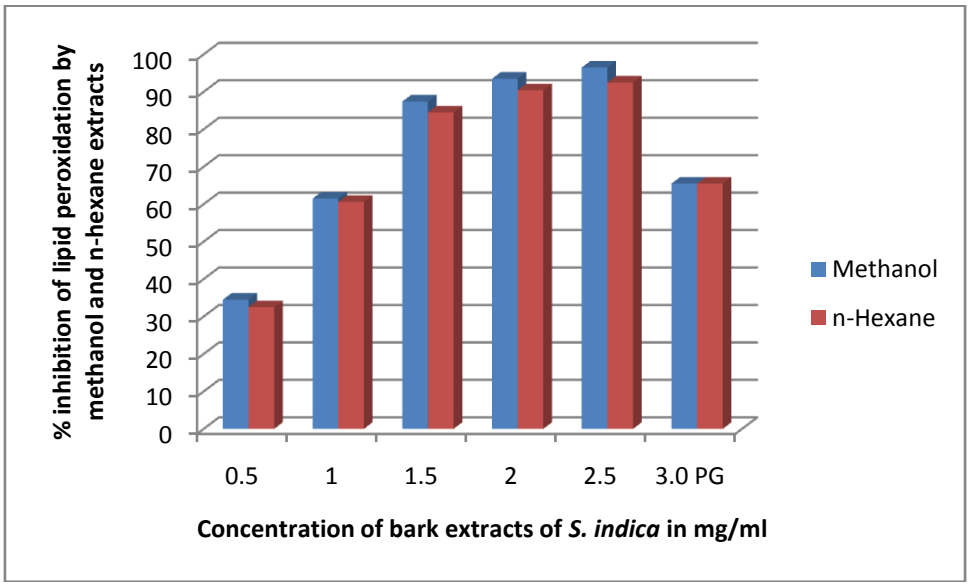

Table-8: % inhibition of lipid peroxidation by methanol and n-hexane extracts of bark of *Saraca indica*

IV. Discussion

In the present investigation, antioxidant activity of the methanol extracts of leaves and bark of *Saraca indica* was assayed in their methanol and n-hexane extracts by total antioxidant capacity, total phenolic content, DPPH scavenging activity, reducing power and lipid peroxidation activity. In all these assays, the antioxidant activity increased with increasing concentration of the extracts of *Saraca indica* (Table-1-4; Fig-1-8). The total methanol and n-hexane extracts showed higher reducing power and percent inhibition of linoleic acid lipid peroxidation considerably than the standard antioxidant *n*-propyl gallate (reducing power activity of IC50 70.25 ± 0.003 and % inhibition of linoleic acid peroxidation of IC50 = 65.5± 0.12 µg/ml). The present findings gains support from the work of Navneet *et al*., (2015) [36] who have observed a more or less similar antioxidant capacity of bark extracts of Saraca indica. Antioxidant activity of plant extracts is not limited to phenolic compounds. The antioxidant activity may also be due to the presence of other antioxidant secondary metabolites, such as volatile oils, carotenoids and vitamins. Thus the present study has shown that the leaves and bark of *Saraca indica* possess significant antioxidant properties and may contribute to the retardation of the inflammatory process. This is because inflammatory tissue injuries are mediated by reactive oxygen metabolites from phagocytic leukocytes (e.g neutrophils, monocytes, macrophages and eosinophils) that invade the tissues and cause injury to essential cellular components (Parfenov, E. A. and Zaikov, G. E. (2000). [37]. Compounds that have scavenging activities toward these radicals have been found to be beneficial in inflammatory diseases (Auddy., et al 2003; Koo et al., 2006) [38, 39]. The ability of the leaf and bark extracts to inhibit the

peroxidation of linoleic acid supports the use of *Saraca indica* in the preservation of palm oil in indigenous societies (Umerie *et al*., 2004) [40]. The antioxidant activity of the extract may also support its traditional use for wound healing. This is because in acute and chronic wounds, oxidants cause cell damage and thus inhibits wound healing (Thang *et al*., 2001) [41]. The administration of antioxidants or free radical scavengers is reportedly helpful, notably to limit the delayed sequale of thermal trauma and to enhance the healing process (Thang *et al*., 2001) [41].

Various scientific studies show that aberrance in redox balance with elevated level of oxygen-free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS) plays an important role in the origin and progression of most human diseases including cancer (Halliwell, 2012; Valko *et al*., 2007; Halliwell , 1991; Cross, *et al*., 1987; Bhattacharyya et al., 2014 (42, 43, 44, 45, 46]. Reactive oxygen species (ROS) act as secondary messenger in intracellular signalling cascades and elevated level of ROS associated with carcinogenesis by promoting initiation, progression, and metastasis of cancer cells. It also induced DNA damage leading to genetic lesions that initiate tumorigenicity and subsequent tumor progression (Volko, 2004; Wang and Yi, 2008; Storz, 2005; Khanna *et al*., 2014 [47, 48, 49]. However, many studies also suggested that free radicals are essential mediators of apoptotic pathway for triggering cell death and therefore function as anticancer agents.Thus, free radicals production approach is used in nonsurgical therapeutic methods for cancer therapy, including chemotherapy, radiotherapy, and photodynamic therapy (Wang and Yi, 2008; Salganik, 2001; Seifried *et al*., 2003) [48, 50, 51]. Free radicals produced in cancer therapy are associated with serious side effects. Furthermore, elevated level of ROS in cancer cell leads to intercellular transfer of hydrogen peroxide (H_2O_2) to neighbouring cells, and stimulates them to acquire uncontrolled ROS production (Storz, 2005) [49]. Free radical scavenger activity plays a protective role in normal healthy cells. They prevent the ROS from spreading and ultimately protect the adjacent cells from oxidative DNA damage and check the cancer progression. Many clinical trials have also suggested that intake of exogenous antioxidants can protect the healthy cells from oxidative stress as well as ameliorate toxic side effects of cancer therapy without affecting therapeutic efficacy (Salganik, 2001) [50]. Extracts of medicinal plants have been used for the treatment of various diseases, including cancer all over the globe, as they are easily prepared, standardized, and stored. Herbal extracts are also cost effective which increase their accessibility to the patients of all economic status (Eder and Mehnert, 1998; Vickers, 2002 [51, 52]. Global health policies promote the therapeutic use of herbal extract.World Health Organization (WHO) also encourages the use ofmedicinal plants in the treatment of disease (Debas *et al*., (2006); Winslow and Kroll, 1998; Pal and Shukla, 2003) [53, 54, 55].

Medicinal plants used as therapeutic agents are considered nontoxic for human consumption, while many studies reported the various side effects of medicinal plant (Chan, 2003; Ergil *et al*., 2002) [56, 57]. Medicinal plants uses for health benefit are not taken under the appropriate instruction and consultant of physician. Although people are using medicinal plants from ancient time, safety evaluation of these medicinal plants are required (Hwang *et al*., 2013) [58].

V. Conclusion

Plants are sources of new natural products used in pharmaceutical, cosmetic and food production. An *in vitro* antioxidant assay provides scientific evidence to prove the traditional claims to the *Saraca indica*. On the basis of the present results it can be concluded that the methanole and n-hexane leaf and bark extracts of this plant possess significant antioxidant activity. Presence of adequate amount of phenolics and flavonoids account for this fact. So the present investigation suggests that *S. indica* is a potential source of natural antioxidant. The active phytochemicals responsible for antioxidant activity and their mechanism of action *in vivo* as well as *in vitro* require further investigation at scientific level.

Conflict of interest: Authors declare no conflict of interest directly or indirectly.

Acknowledgement: Authors are thankful to Dr. Baidyanath Kumar, Department of Biotechnology, Patna Science College (Patna University), Patna for providing necessary suggestion and support

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Dr Baidyanath Kumar, et. al. "In Vitro Antioxidant activity of leaves and stem bark of Saraca indica L." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 15(5), (2020): pp. 52-64.