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Evaluation of Secondary Metabolites and Determination of Antioxidant Activity of Indigofera Trita

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Abstract

To quantify the major secondary metabolites and the antioxidant potential of methanolic extract of *Indigofera trita*. The methanolic whole plant extract of *Indigofera trita* (MIT) was analaysed by HPLC and GC to determine various Phytochemicals. Free radicals scavenging activity of extract by using DPPH, NO and Super oxide radicals generated *in vitro*. The Methanolic extract of *I. trita* was found to contain alkaloids (1.55gm/gm), terpenoids (0.98mg/gm), phenols (6.27mg/gm) and flavonoids (1.007mg/gm). The major flavonoid detected was quercetin and rutin. The MIT was found to possess significant radical scavenging activity against DPPH, No and superoxide anions the IC_{50} value of 52.0 µg/ml, 52.0µg/ml and 52.6µg/ml respectively and comparable to that of their corresponding IC_{50} value. The medicinal property of *Indigofera trita* may be attributed to the presence of Flavonoids and phenolic compounds with rich antioxidant potential. The therapeutic effect of this plant may be accounted for its counteracting action on free radicals *in vivo*.

Key wards: *Indigofera trita*, Phytochemicals, free radical, scavenging activity

Introduction

Natural therapy for various human ailments purified with plant products has gained much attention now days, due to various side effects associated with allopathic medicine these can be derived from any part of the plant like bark, leaves, stem, flowers, roots, seeds, etc., [1]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects [2].

Free radicals play an important role in various pathological conditions such as tissue injury, inflammation, neurodegenerative diseases, cancer and aging. The Compound that can scavenge free radicals has great potential in ameliorating these diseases [3]. Inflammation is a disorder characterized by invasion of leucocytes and production of proinflammatory cytokines [4].

Indigofera trita commonly known as punalmurunkai and kattuavuri is widely distributed in India, Ceylon, South Africa and North Austrilia [5]. The entire plant is traditionally used by tribes and native medicinal practioners to treat diseases such as inflammation, tumor, liver diseases [6] Literature review revealed that the plant *I. trita* is having anti-tumor [7] hepatoprotective, anitioxidant [8] Based on above details the present study is aimed to investigate the antioxidant activity of MIT by using in vitro models of free radicals.

Taxonomy

Fig 1 : *Indigofera trita* linn

Kingdom	:	Plantae
Order	:	Fabales
Family	:	Fabacea
Genus	:	Indigotera

Materials and Methods

Collection and Identification

Indigofera trita was collected from Ammapettai in Thanjavur District. The plant was authenticated by Dr.P.Jayaraman, Director, Plant anatomy &research center, West Tambaram, Cheennai- 45. (PARC/2015/3042) and the voucher specimen is deposited in our laboratory.

Preparation of methanolic extract of *Indifgofera trita*

The whole plant was shade dried and pulverized 100gm of the powder was soaked in 150ml of methanol (w/v) for 3-5 days with intermediate shaking. This was filtered through a fine cheese cloth and the filtrate was pooled after 3 days of repeated extractions. The filtrate obtained was evaporated to dryness using rotary evaporator. The concentrate was lyophilized and used for the study.

HPLC – UV analysis (Total Phenols)

MIT was subjected to solid phase extraction using column 5mm (4.6.mm) & peptides, small molecules were

removed fractionation of neutral and acidic phenolic acids was also carried out simultaneously. The resulting fraction was then subjected to reverse phase high performance liquid chromatography (RP-HPLC). The total phenolics in MIT was detected using, Stationary phase octadecylsil. Silica and mobile phase (A phosphoric acid: water (0.5: 99.5v/v) B acetonitrile). The UV detector was set at 220 nm with the flow rate adjusted to 1.0ms / min. The major peaks were identified and the retention times were compared with these of standards.

Fractionation of total Alkaloids

MIT was detected using monobasic Phosphate as mobile phase (270ml. of Acetonitril). The liquid Chromatography is equipped with 235 nm detector & 4.6nm x 150 mm column. The flow rate was adjusted to 1.8ml / minute the major peaks were identified and the total alkaloids concentration were determined.

Fractionation of total Flavonoids

HPLC Chromatography (System Name: LACKROM L-7000 MERCK, Proc Method – HITECHI) total flavonoids. The total flavonoids in the extract was determined by using octadecysil silica gel as stationary phase and acetonitril, sodium dihydrogen phosphate with dilute orthophosphoric acid as mobile phase. UV detector was set at 350nm with flow rate of 0.5ml/min. The major peaks in MIT were determined in comparison to the retention time of standards run at identical conditions.

Gas chromatography (GC analysis of terpenoids)

The terpenoids level was measured GC using capillary column coated with macrogol 20000R and nitrogen as carrier gas. The flame ionization detector was set at the flow rate of 0.4ml/min & used as standard.

Free radical scavenging activity

1. Diphenyl – 2- Picrylhydrazyl (DPPH) radical scavenging activity.

DPPH radical scavenging assay is a commonly recommended method for assessment of antioxidant potential of plant extracts. The assay is based on the ability of DPPH, a free radical which get decolorized in the presence of antioxidants. To 200ml. of methanolic solution of DPPH (1 µg/ml) various concentration of (20mg – 100 µg/ml) in water were added and incubated at 370C for 30 min in dark and the absorbance was measured at 517nm. Ascorbic acid was used as the reference standard. The percentage scavenging of DPPH free radical was calculated and compared with that of the standard ascorbic acid. The IC50 value also determined.

Superoxide anion scavenging activity [9]

The method was applied for the measurement of MIT superoxide anion scavenging activity, Briefly 312µm Nitroblue tetarzolium in 120 µm phosphate buffer 74 were added to an aliquots of MIT (20-100µg/ml) the reaction was started by adding 100ml of phenazinemethosulphate (120mm preaperd in phosphate bufferpH 7.4) and the colour change was monitored at 560nm against water blank querceutin was used as the positive control.

Nitric oxide scavenging activity

The nitric oxide scavenging activity of the aqueous extract was measured by taking various concentrations of MIT and standard. Ascorbic acid (20-100µg/ml) dissolved in phosphate buffer (0.025m, pH 7.4) and incubated with sodium nitroprusside (5mm) in standard phosphatebuffer at 25^0C for 5 hrs. After the incubation, 0.5ml of the reaction mixture was added with 0.5ml of Griess reagent (equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% napthlthyl ethylene diamine dihydrochloride in water). The absorbance of the chromophore formed was read at 540nm. The activity was compared with that of similar concentration of Ascorbic acid [10].

Result and Discussion

Preliminary phytochemical screening of methanol extract of *Indigofera trita* revealed the presence of alkaloids, flavonoids, terpenoids and phenolic compounds which are essential to prevent diseases and to maintain a state of well being. Recent studies have been focused on finding the natural substance of medicinal plant that decrease the inflammation and reduce oxidative stress and there by counteracting the macromolecular damage. It is well known that reactive oxygen species interact with key bimolecular such as proteins and enzymes which regulate major metabolic path way and decrease their functional efficiency.

Table – 1
Quantiative Phytochemical Analysis

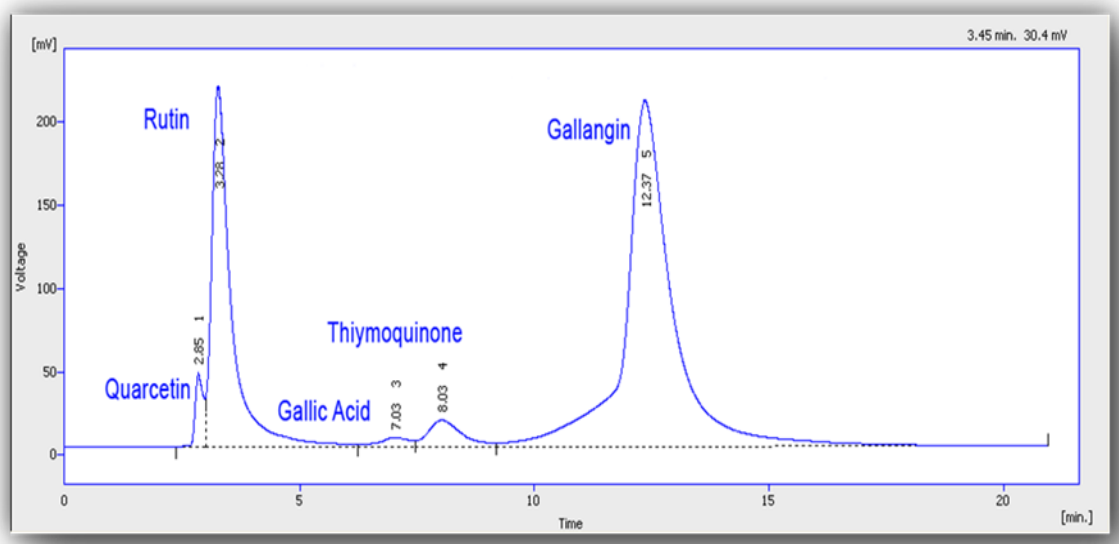
S. No.	Phytochemicals	Quantity mg/gm of dry material
1.	Alkaloids	1.55
2.	Terpenoids	0.98
3.	Total phenols	6.27
4.	Gallic acid	5.34
5.	Cinnamic acid	0.51
6.	Coumaric acid	0.42
7.	Flavonoids	1.007
8.	Rutin	0.244
9.	Quercetin	0.763

Shows that MIT contains [11] rich amount of bioactive compounds which exhibit antioxidant property the quantitative analysis revealed that MIT contain rich amount of phenolic compounds and flavonoids. It is well known that

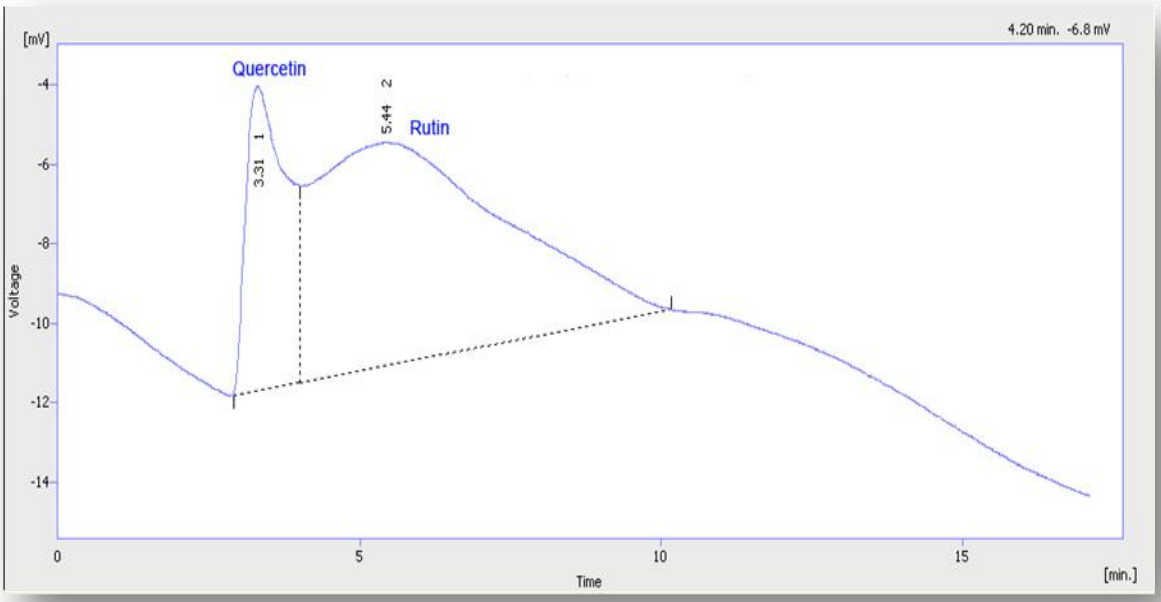
plant flavonoids and phenols in general are highly effective in scavenging free radical and providing antioxidant defense in living cells. Ployphenols and flavonoids isolated from plants medicinal have been used for the prevention and cure of various diseases which are mainly associated with free radicals.

HPLC Analysis of MIT

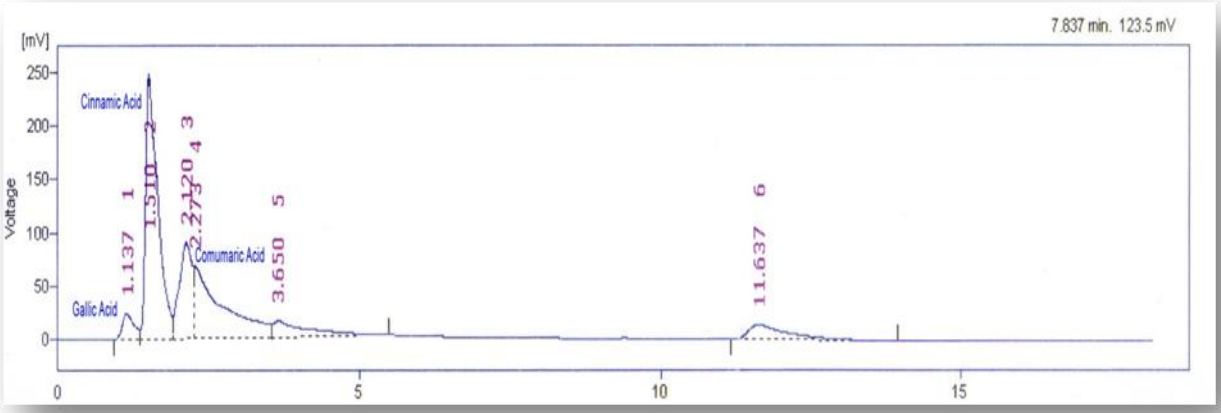
HPLC analysis reveals that the extract was found to be rich in Alkaloids (1.55 mg/g) terpenoids (0.98mg/g) and phenols phenolcs (6.27 mg/g). MIT also contain flavonoids such as Rutin (0.244mg/g) and quercetin (0.763mg/g) Fig. 2 (A) & (B) many reports demonstrate that antioxidant principle present in medicinal plants are responsible for their therapeutic potential[12]. The flavonoic compound such as quercetin and Rutin are formed to be responsible for anti-inflammatory and anticancer properties proliferate by their terminating action of free radicals [13]. Alkaloids have many pharmacological activities including anti cancer& anti arthythmic effect [14]. Alkaloids are known to reduce the inflammation level significantly. This results shows that MIT containing which could be accounted for the antioxidant and anti-inflammatory effects.



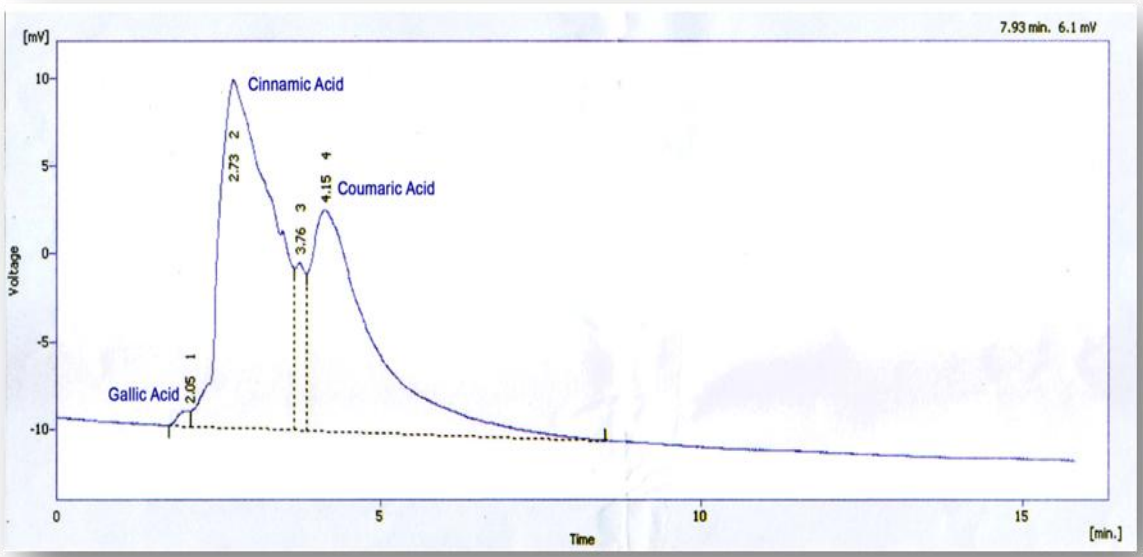
(A) HPLC Finger prints of standard flavonoids.



(B) HPLC Finger prints of Flavonoids present in MIT.



(C) HPLC Finger print of standard Phenols



(D) HPLC Finger print of Phenols Present in MIT.

It may lead to oxidative stress. The Natural phytonutrients presents in fruits and vegetables scavenge the free radicals and protect the cells from oxidative damages. The phytonutrients present in MIT migrates the responsible for the traditional claim by the test drug.

Reactive oxygen species and free radicals known as super oxide anions, hydroxy radicals, hydrogen peroxide are the major class of highly reactive species derived from normal all metabolism of major nutrients[15]. These highly reactive free radicals if not counteracted and inactivated by cellular antioxidants.

The DPPH is decolourised nature it receives electron or hydrogen atom from antioxidants and the extend of decolorisation represents the antioxidant potential of the test compounds. The result obtained in these investigation shows that MIT possess a potent scavenging activity against DPPH radicals (Fig.4) The scavenging activity was comparable to that of standard ascorbic acid.

The IC50 value of MIT (52.0µg/ml) was found to be nearer to that of standard ascorbic acid (55µg/ml) supe oxide anion scavenging activity.

Calculate the percentage inhibition = (optical density of Control- Optical density of Test / Optical density of Control) x 100

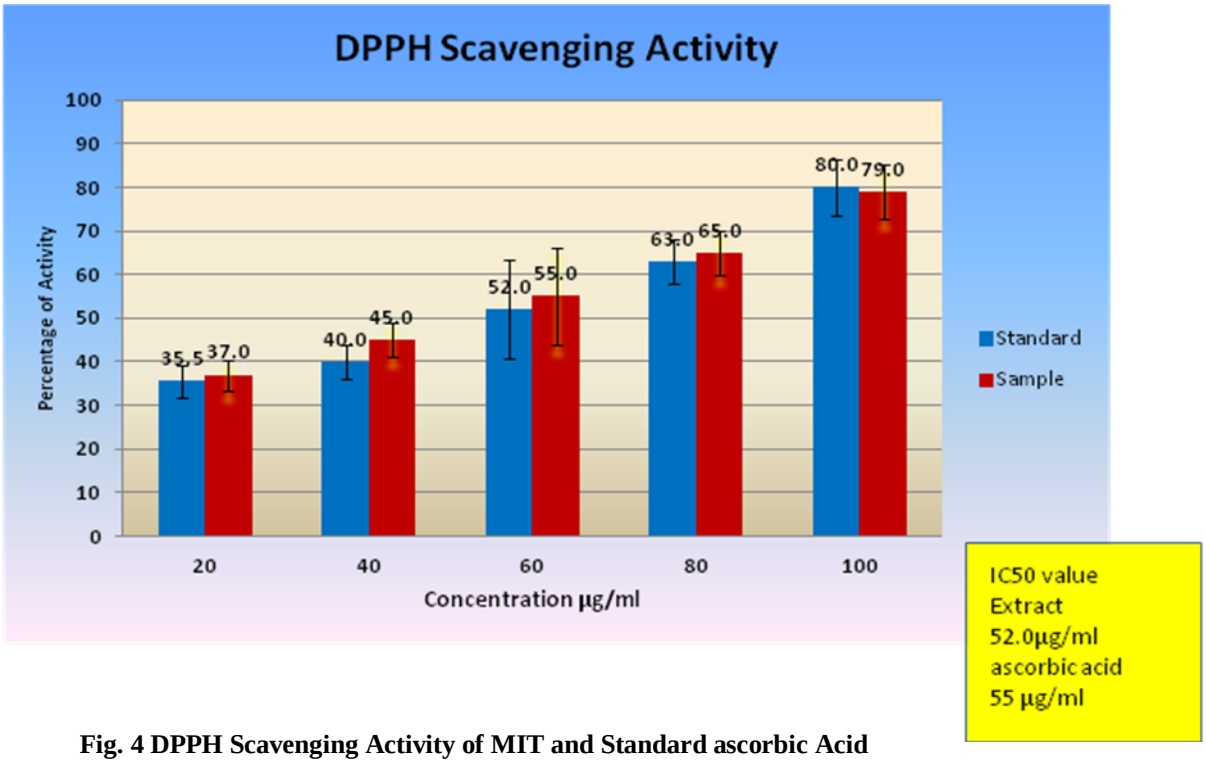


Fig. 4 DPPH Scavenging Activity of MIT and Standard ascorbic Acid

Super Oxide Anion Scavenging Activity of MIT was found to possess comparable free radical scavenging activity against super oxide anions when compared to that of standard quercetin (Fig.5). The IC_{50} value was found to be (52.0 ug / ml and in 61.9ug/ml) for MIT respectively.

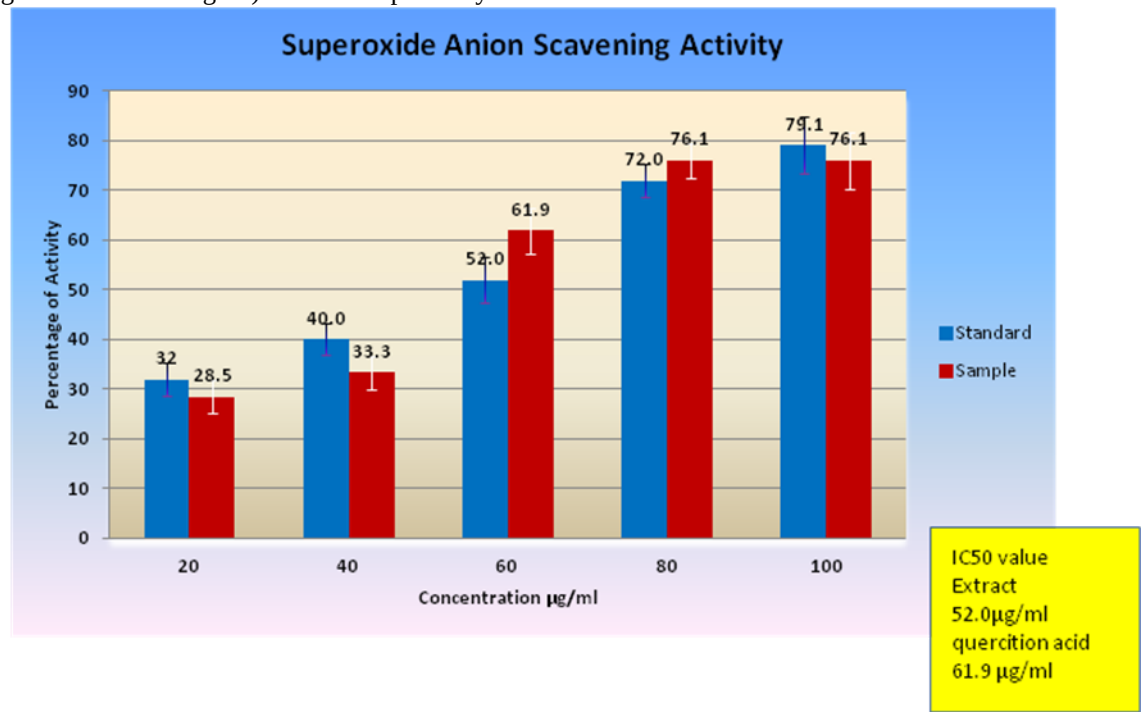


Fig. 5 super oxide anion scavenging activity of MIT and standard quercetin.

Calculate the percentage inhibition = (optical density of Control- Optical density of Test / Optical density of Control) x 100

The superoxide onions are toxic intermediates formed during inflammatory process and found to enhance the risk of inflammation related disorders such as arthritis and atherosclerosis. Super oxide anion is a free radio that plays an important role in the formation of reactive oxygen species such as hydrogen periode, hydroxyl / radicals, or singlet oxygen in living organism. Reported that the therapeutic activity of medicinal plants can be determined by superoxide activity [16].

Nitric Oxide scavenging activity is on important chemical mediator generated by endothelial cells, macrophages, neuron & it is involved in the regulation of various physiological process like control of arthritis, cytotoxice effects Alzhemer's disease [17]. No formation is toxic to living organism and it was found that MIT significantly scavenges the

nitric oxide and the effect was comparable to that of standard Ascorbic acid at similar concentration with IC_{50} value (52.6µg/ml and 56.8 µg/ml) of and respectively.

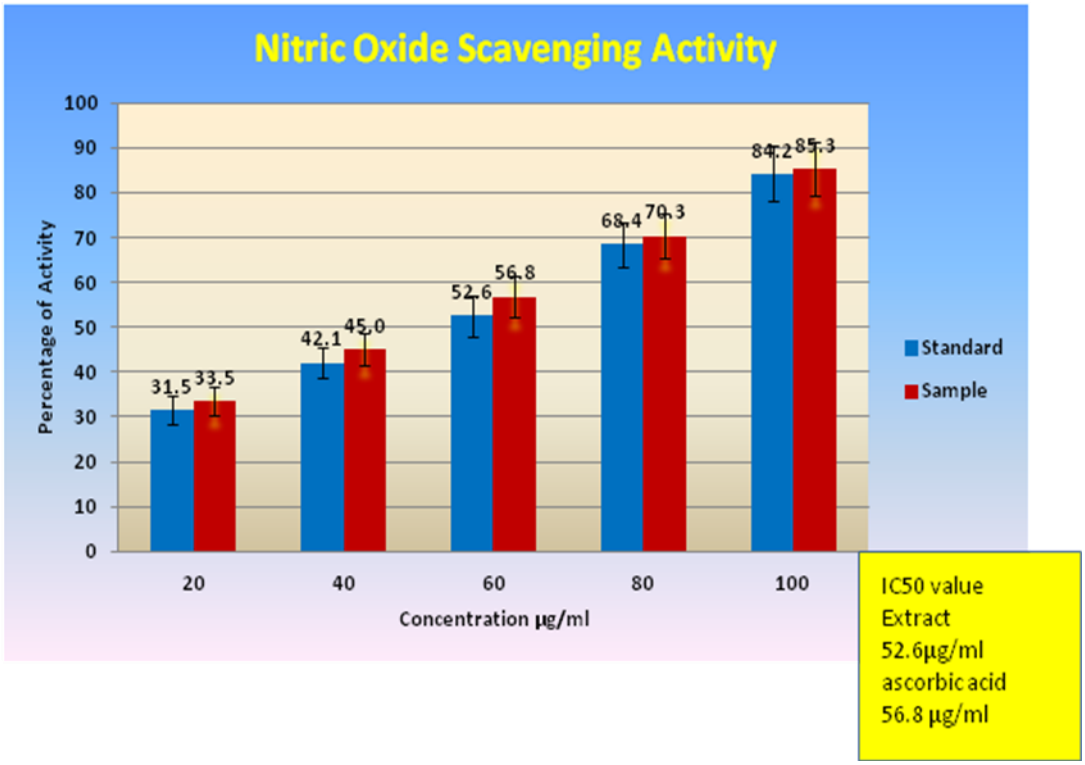


Fig. 5 Nitric oxide anion scavenging activity of MIT and standard Ascorbic acid.

Calculate the percentage inhibition = (optical density of Control- Optical density of Test / Optical density of Control) x 100

The result of preliminary phytochemical screening shows the presence of flavonoids such as Quercetin and Rutin, Phenolic compounds and Alkaloids in the Plant. A large number of these compounds are known to possess strong antioxidant properties. The free radical scavenging activity of *Indigotera trita* revealed that they can be used for the prevention or treatment of human diseases such as cancer, arthritis, diabetes mellitus which are associated with oxidative stress.

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