



In Vitro antioxidant, anti-inflammatory potential of *Piper nigrum* fruit and *Anacyclus pyrethrum* root extract

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ABSTARCT

Piper nigrum belongs to the family piperaceae is known as king of spices it has various pharmacological activities. *Anacuclus pyrenthrum* belongs to the family of asteraceae it is one of the traditional medicinal plants it has lots of uses. The main study of this research is the *in vitro* antioxidant anti-inflammatory and anticancer activity of *piper nigrum* and *Anacyclus pyrenthrum*. 5 g of samples were separately soaked in 100 mL of ethanol under cold condition for 24 hrs and compounds extracted by ultra-sonification at 450Hz for 5 cycle and dry them in a heat sterile. GC-MS assay are done for the identification of phytochemical analysis. A biomedical application such as antioxidant assay was done by DPPH radical scavenging mixed with a different ratio with an increased protection of 42-45% and metal chelating shows an increased protection of 70-85%. Anti-inflammatory HBRC assay showed a higher inhibition of 85-90%. *In-silico* anticancer activity done by molecular docking with the protein human estrogen alpha receptor shows a maximum binding score of -6 to -7. The present study result showed the *Piper nigrum* and *Anacyclus pyrenthrum* a good source of antioxidant, anti-inflammatory and anticancer analysis.

Key words: *Piper nigrum*, *Anacucluspyrenthrum*, antioxidant anti-inflammatory, anticancer activity, GC-MS assay, DPPH.

Received 24.10.2022

Revised 16.11.2022

Accepted 28.12.2022

INTRODUCTION

Piper nigrum (*P. nigrum*) is commonly used in traditional medicine. There is an increasing recognition that many of today's diseases are due to the "oxidative stress" that results from an imbalance between the formation and neutralization of reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can be removed with antioxidants. Antioxidants are widely used as ingredients in dietary supplements and are exploited to maintain health and prevent oxidative stress-mediated diseases such as cancer, atherosclerosis, diabetes, inflammation and ageing [1]. Many antioxidants have been isolated from different plant materials. The main objective of the present study was to evaluate the antioxidant activity of two different medicinal plants used.

Piper nigrum (family Piperaceae) is a valuable medicinal plant. It is one of the most commonly used spices and considered as "The King of spices" among various spices. Pepper is grown in many tropical regions like Brazil, Indonesia, India, Malaysia, Vietnam, and Sri Lanka. Pepper which is the most famous and one of the commonly used spices throughout the world. The plant can reach up to 50–60 cm in height and is characterized by its simple, alternate leaves, with a few rare cases of opposite or verticillate leaves [2]. The most commonly used part of the plant is the aromatic fruit. Pepper is used worldwide in different types of sauces and dishes like meat dishes. Interestingly, white, green, and black peppers are products of the *P. nigrum* fruits at different ripening stages [3]. Whole Peppercorn of *Piper nigrum* or its active components are being used in different types of foods and as medicine. Traditionally, pepper has been used in many Asian countries for treating indigestion, asthma, pain, respiratory tract infections, and rheumatoid arthritis. It is also a stimulant, digestive, tonic, and antiseptic [4]. Antioxidant, antitumor, antipyretic, analgesic, anti-inflammatory, antidiarrheal, antispasmodic, hepato-protective, antibacterial, antifungal, anti-thyroids, anti-apoptotic, anti-spermatogenic, insecticidal and larvicidal activities [5] *Anacyclus pyrethrum* (*L*) is commonly known as African pyrethrum, akarkarha, tigendesste, and igendess. This species includes two varieties *Anacyclus pyrethrum* var. *pyrethrum* (*L*) and *Anacyclus pyrethrum* var. *depressus* (*Ball*) *Maire* [6-7]. In addition, this plant is prescribed for treating partial paralysis of the tongue and lips, gout, sciatica and the root of this plant are stimulant, cordial and rubifacient. A gargle of infusion

is prescribed for relaxed vulva and also used for rheumatic, neuralgic affection and rhinitis and it decrease the plasma glucose and serum cholesterol level after administration for 3-6 week[8].

Antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule. The main characteristic of an antioxidant is its ability to trap free radicals [9]. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Herbal plants considered as good antioxidant since ancient times [10]. Plants are important source of antioxidants. Antioxidants completely stop or delay the process of oxidation. Some in vitro studies revealed that piperine inhibited free radicals and reactive oxygen species, therefore known to possess protective effects against oxidative damage [11]. *Anacyclus pyrethrum* extracts (aqueous or methanolic) produces similar antioxidant activity, especially in DPPH (2,2- diphenyl-1-picrylhydrazyl) test Phytochemicals like Phenol, Flavonoids, Alkaloids and Tannins[13-14].

MATERIAL AND METHODS

Collection of Samples

Fresh Green *Piper nigrum* collected from yercaud hill station and *Anacycluspyrethrum* (Akkarakaram) root were commercially obtained from herbal shop near Malaikotai, Tiruchirappalli .5 g of samples were independently soaked in 100 mL ethanol under cold condition for 24 h and compounds extracted by ultrasonification at 450Hz for 5 cycles. The extract was filtered and evaporated to dryness under vacuum in a rotary evaporator.

Preliminary phytochemical screening:

Qualitative phytochemical analysis was performed by using appropriate procedures by Thanh-Tam [15], Leonardo Hanane [16].Qualitative phytochemical analysis was carried out to identify the secondary metabolites present in the Ethanol extracts of *Piper nigrum*green fruit and *Anacyclus pyrethrum*.

TLC:

The high sensitivity of TLC is used to check the purity of the samples. With the help of TLC, it is possible to know whether a reaction is complete and had followed the expected course. The homogeneity of the compounds was monitored by this TLC plates and visualized by iodine vapour.

Separation : 10 µL sample is applied on pre prepared TLC plates (MREK) activated at 100° C for 5 min and then placed on a beaker contain mobile phase. A glass chromatographic tank saturated with the mobile phase for 30 min was used for linear ascending development. The developed plates were air dried and then UV scanned at 275 nm.

Preparation of extract formulation

Fixed volume of green pepper extract was taken in 7 tubes and different concentration(V/V)of akkarakara extract is add to prepared 100:10 to 100:100:50 ratio and vice versa concentration of akkarakara and green pepper extract.

2,2-DIPHENYL-1-PICRYL-HYDRAZYL (DPPH) REDUCTION ASSAY

A method that uses free radical traps to assess the antioxidant capacity of the samples. DPPH is a stable free radical due to the delocalization of the spare electron over the molecule as a whole, with a deep violet color characterized by an absorption band at 520 nm. When DPPH is mixed with an antiradical compound that can neutralize it, it becomes colorless. Therefore, the decrease in optical density of DPPH radicals is monitored to evaluate the antioxidant potential of the samples.1,1-Diphenyl-2-picrylhydrazyl (DPPH) 100 µL of different concentration of above prepared herbal mixture were taken in 1 ml ethanol.0.2 mL of DPPH reagent. The DPPH reagent was added to each well in dark environment [17]. Then, the plate was wrapped in aluminum foil and incubated on the shaker at room temperature for 20 minutes. The absorbance was recorded at 517 nm. Ascorbic acid used as standard. % Of DPPH inhibition = C-T/CX100.

DETERMINATION OF METAL CHELATING ACTIVITY

Metal chelating activity was measured as described previously, by adding 0.1 mM FeSO₄ (0.2 mL) and 0.25 mM ferrozine (0.4 mL) subsequently into 0.2 mL of extract at different concentration. After incubating at room temperature for 10 min, absorbance of the mixture was recorded at 562 nm. **EDTA used as Standard.** Chelating activity was calculated using the following formula:

Metal chelating activity = (A control – A sample)/A control x 100

RESULTS AND DISCUSSION

Extraction and phytochemical analysis

Piper nigrum green fruit and *Anacyclus pyrethrum* (fig.1) powdered were extracted with ethanol. The extract of green pepper is red in color and *Anacycluspyrethrum* pale yellow in colour having pH 6-7 (Fig.2). Table 1 list the presence of different phytochemical detected from the extract by qualitative

phytochemical analysis(Fig3-4).The *P.nigrum* fruit shows presence of Alkaloids, Flavonoids, Glycoside, Tannins and *Anacyclus pyrethrum* shows the occurrence of flavonoids phenol, tannins and coumarin. Likewise, our results belonging to the phytochemical constituents of peppers extracts agreed the presence of glycosides described by Jyothiprabha and Venkatachalam 2016 from different solvent extracts of pepper fruits. Phytochemical screening has identified various secondary metabolites such as alkaloids, reducing compounds, tannins, flavonoids and coumarins[18]. Chemical analysis of roots shows the presence of three fatty acids, a sterol and ten unsaturated amides. The most important compounds discovered in roots are pellitorin, anacyclin, phenylethylamine, inulin, polyacetylenic amides I-IV, and sesamin. The species contains also tannins, gum and essential oil traces

PLANT SAMPLE USED



Fig.1 *Piper Nigrum Anacyclus Pyrethrum*

EXTRACTED PHYTOCHEMICAL



Fig.2: Extracted phytochemical

Table 1 Qualitative phytochemical analysis

TEST	Green pepper fruit extract	Akrakara root
Alkaloids	Present	Absent
Flavonoids	Present	Present
Sterol	Absent	Absent
Phenol	Present	Present
Glycoside	Present	Absent
Quinones	Absent	Absent
Tannins	Present	Present
Carboxylic acid test	Absent	Absent
Saponin	Absent	Absent
Courmarin	Absent	Present

QUALITATIVE PHYTOCHEMICAL TEST

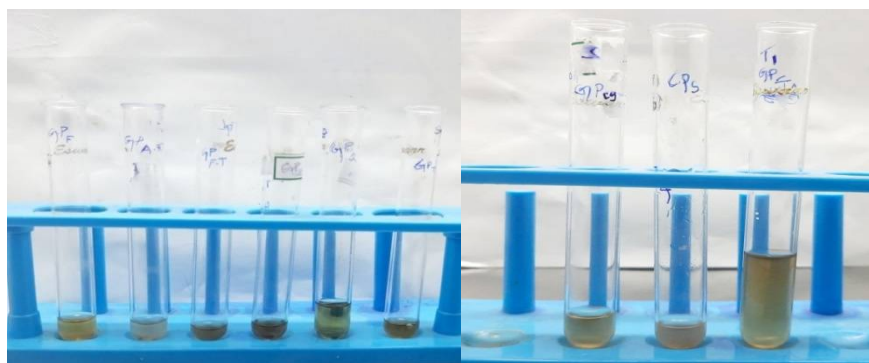


Fig.3 Phytochemical test (*P. Nigrum*)

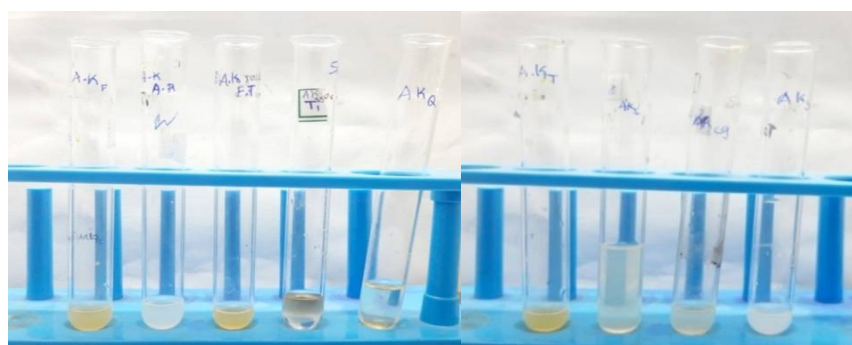


Fig.4 Phytochemical test (*A. Pyrethrum*)

THIN LAYER CHROMATOGRAPHY

TLC is a highly versatile separation method that is widely used for both qualitative and quantitative sample analysis. The extraction of green pepper fruits and akkarakaram was performed and crude extract were placed in TLC plate. Both the pepper and akkarakaram were fixed on TLC plate shows single fraction under the UV 350 nm as well as iodine vapor (Fig.5). Iodine fuming and UV light were found to be a convenient visualizing agent. The R_f value of piperine was recorded as 0.73 and for *Anacyclus pyrethrum* is 0.75 cm.

Because of the mixtures of ingredients in traditional medicinal preparations, TLC separation of piperine from other chemical components was a challenge. Many mobile phases previously reported were assessed and dichloromethane-ethyl acetate 75:10 resulted in the best separation on silica gel TLC plates. The composition of this mixture was adjusted to 9:1 for better resolution. The R_f value of piperine was 0.38[19].

THIN LAYER CHROMATOGRAPHY

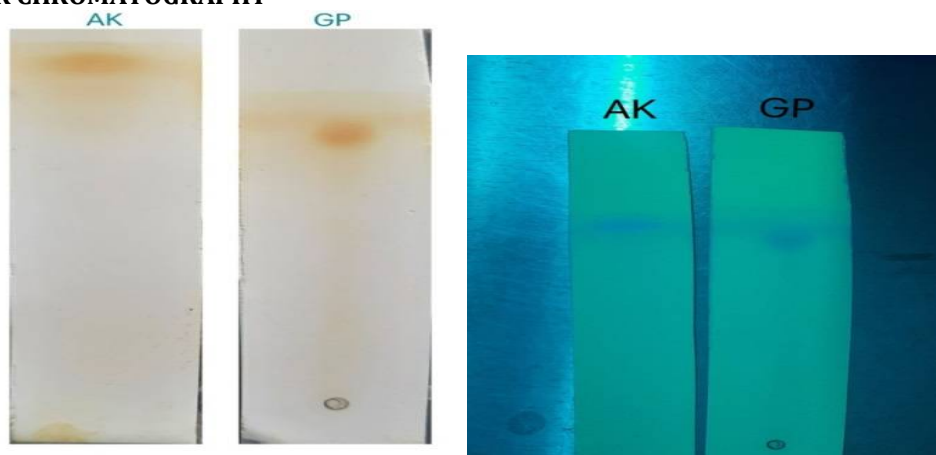


Fig.5 TLC with iodine vapour TLC under ultra violet rays

Table 2 Rf Value Calculation

SAMPLE	Rf Value	Iodine
<i>Piper nigrum</i>	0.73	0.73
<i>Anacyclus pyrenthrum</i>	0.75	0.75

ANTIOXIDANT ASSAY

The scavenging of DPPH radical was evaluated by absorbance of antioxidant at 517nm (plate 5). Synergistic antioxidant of *P.nigrum* with different concentration of akkarakaram were represented on figure 2. The DPPH scavenging of *P.nigrum* alone was 21 % and the activity varied by the addition of akkarakaram and found to be increased up to 42 % at 1:0.5 ratio (GP/AK) followed by 33.4% at 1:0.3 where least activity of 9.5% recorded at 1:0.1. from this ration the green pepper found synergistically active at 1:0.5ratio. The herbal mixture of *Anacyclus pyrethrum* extract with different volume of *P.nigrum* at different ratio revealed the similarity antioxidant activity (figure 3).In this study the combination of herbal mixture varied, and least activity recorded as 9.5%at 1:0.1 and 1:0.2 and moderately 33 and 37 % at 1:0.3 and 1:0.4. the activity was significant at 1:05 calculated as 45%. An increase in radical scavenging activity was noted in AK when added with *P.nigrum* raised to 45% from 19 % .Ascorbic acid kept as standard and the percentage were noted as 61%.

Studies conducted by green peppers reached 30-34 % antioxidant respectively at a concentration of 1500 µg/mL, previously reported by Mennat [20]. The DPPH radical is commonly used to determine the antioxidant activity as a substrate. The DPPH is a stable free radical to become stable molecule that can accept a hydrogen radical or electron from antioxidant substances found in examined samples Taher.The ethanolic extract of *Anacyclus pyrethrum* and its Antioxidant potential of *A. pyrethrum* root may be due to their photochemical constituents such as Phenol, Flavinoids, Alkaloids, Tannins is reported earlier by *Fatima Zahra* [21]. Previously reported by Park[22] who found that yellow pepper extract had the highest antioxidant IC50 = 811.09 µg/mL followed by red pepper extract with IC50 = 882.31µg/mL.

ANTIOXIDANT ASSAY BY DPPH

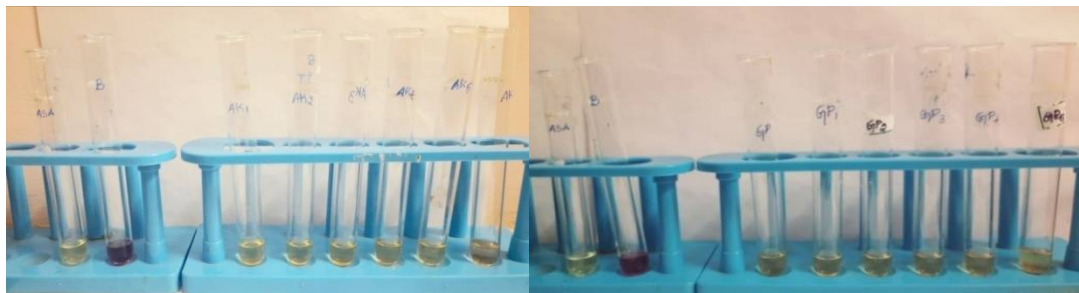


Fig.6: DPPH test of *A. Phyrenthrum* Fig.7 DPPH test of *P.Nigrum*

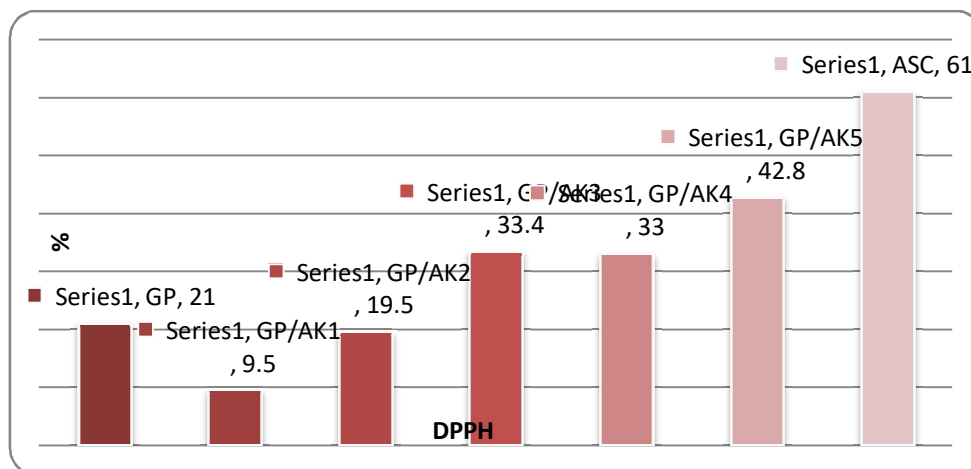


Fig.8 Percentage of DPPH scavenging of GP and different con of akkarakara

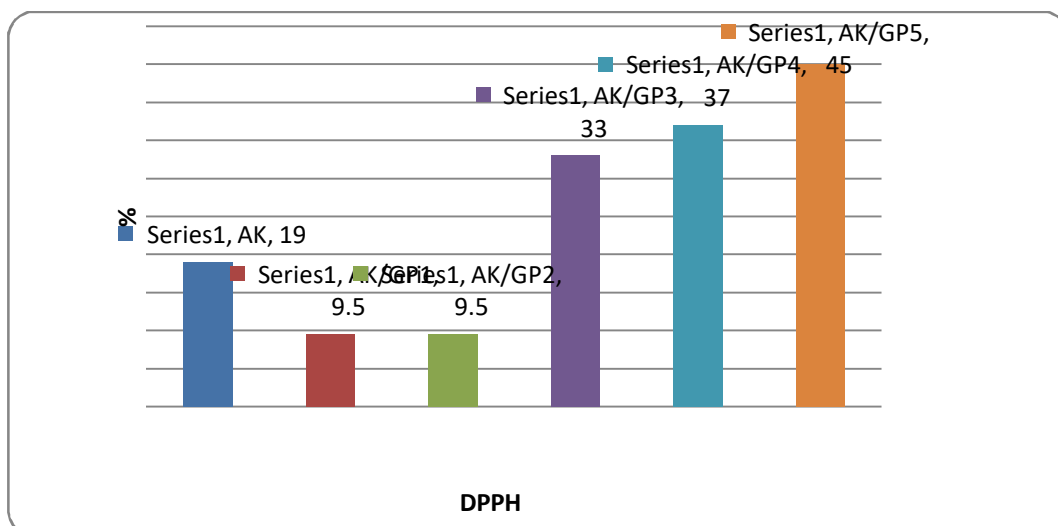


Fig.9 Percentage of DPPH scavenging of akkarakara with different con of green pepper

METAL CHELATING

Metal chelating activity was measured using different concentration of the extract whether the extract able to chelate the metal ion tested at different concentration(25-100µg) and compared with standard EDTA. Figure 4 represent metal chelating potential of *P.nigrum*. Data reveals that extracts increasing concentration reduce the metal chelating property. Maximum activity was noted at 25µg and recorded as 84% followed by 70% at 50µg/mL. The acitivity decreased at 75 and 100 µg (less than 30%) . The percentage of metal chelating in *A.Pyrethrums* also found similar chelating activity and recorded as 81.82, 79.54, 63.63 and 47.72%respectively among 25- 100µg (figure 5).In bith extract there is no significant metal chelation was observed above 50µg/mL. Metal chelating potency of phenolic compounds is dependent upon their unique phenolic structure and the number and location of the hydroxyl groups [23]. Metal chelating ability of water and ethanolic extract of black pepper was reported as 84% respectively.

METAL CHELATING ASSAY

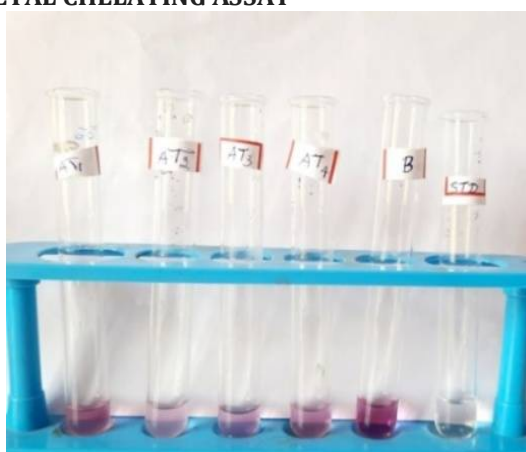


Fig.10 *A.Pyrethrum*

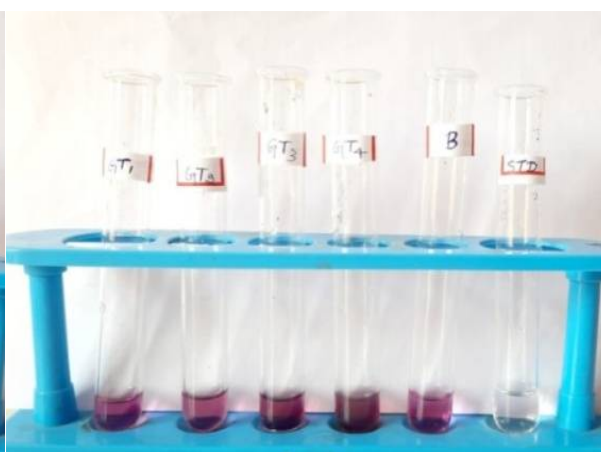


Fig.11 *P.Nigrum*

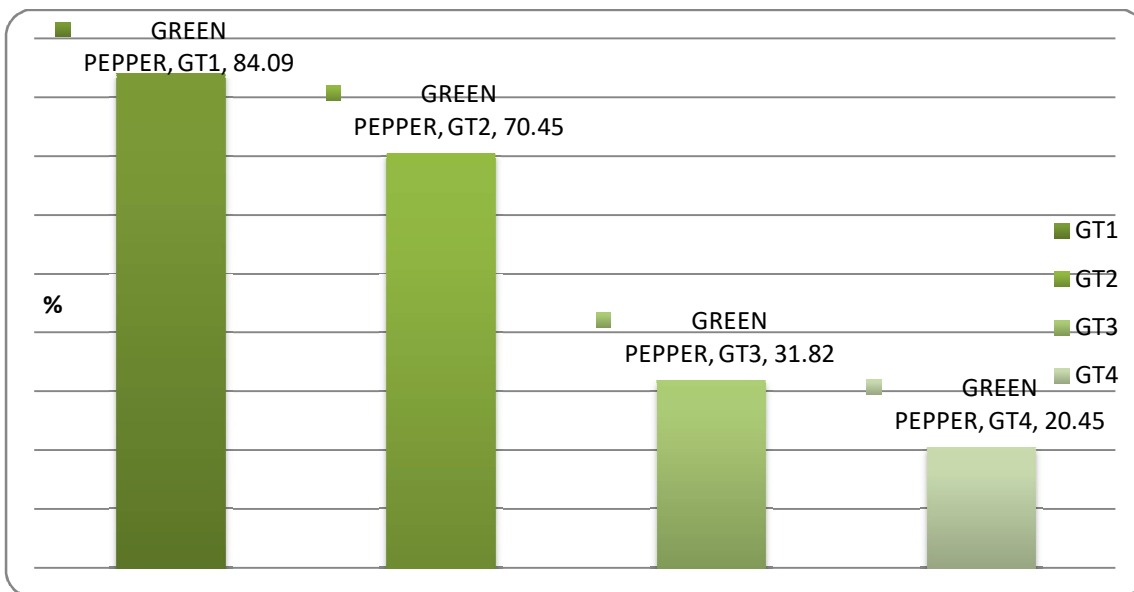


Fig.12 Green Pepper metal chelating activity

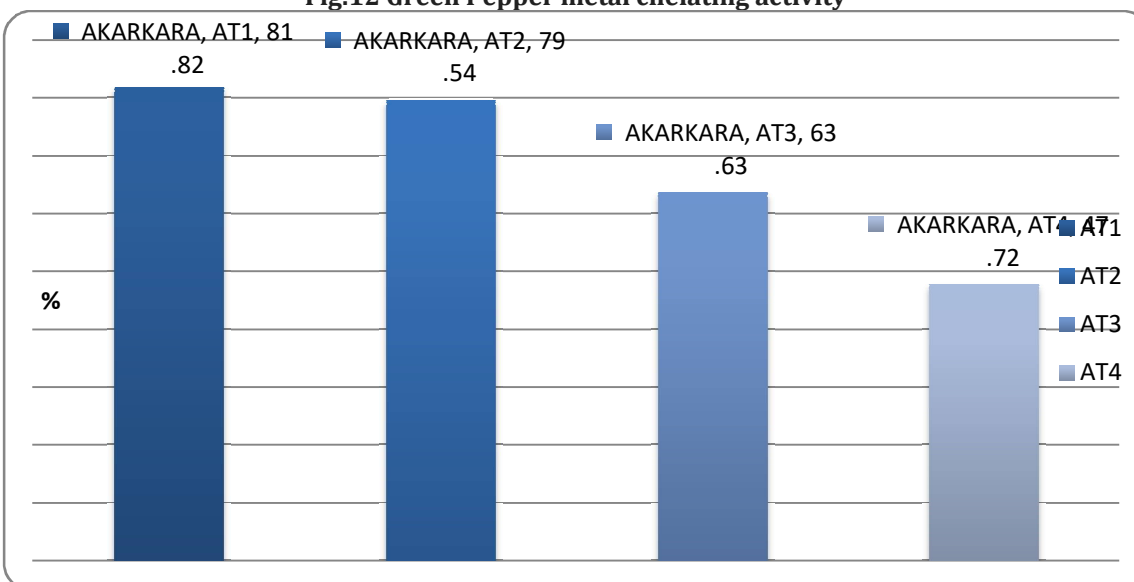


Fig.13: Akarkara [Metal Chelating]

ANTIBACTERIAL ASSAY

The *Piper nigrum* and *Anacyclus pyrethrum* extract showed a significant antibacterial activity against *P.aeruginosa* determined by well diffusion method(plate 8) the antibacterial activity of *P.nigrum* shows 24 mm zone of inhibition at 100 µg and less than 10 mm at 50µg, likewise the extract of *Anacyclus pyrethrum* shows moderate activity with maximum 16 mm inhibitory zone against test pathogen organic extracts of black pepper possess good inhibitory activity against both gram positive and gram negative bacterial strains. According to Harold [24] the antimicrobial activity of black pepper is due to the presence of essential oil (3%), whose aroma is dominated by monoterpenes hydrocarbons: sabinene, β-pinene and limonene. Furthermore, terpinene, α-pinene, myrcene, and monoterpene derivatives like borneol, carvone, carvacrol, 1,8- cineol and linalool are also present. Selles[25].showed that the essential oil from Algerian *Anacyclus pyrethrum* L. has activity against *Candida albicans* and *Staphylococcus aureus*. Studies on biologically active substance of *Anacyclus pyrethrum* and its antibacterial effects are very rare. It has not been elucidated that which active component or either biologic mechanism of *Anacyclus pyrethrum* has antibacterial activity on *E. coli*.

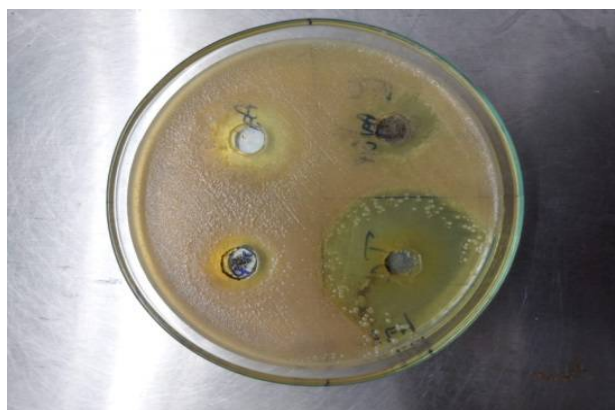


Fig.14 ANTIBACTERIAL TEST

CONCLUSION

In the present investigation, a sustainable novel therapeutic agent from natural sources and their possible intervention studies has been one of the major areas in biomedical research in recent years. Piper species are highly important - commercially, economically and medicinally. *Anacyclus pyrethrum* an amazing medicinal plant is one of the most widely growing species of the family Asteraceae. *Anacyclus pyrethrum* (L.) is a plant widely used in medicine to treat inflammatory and painful diseases. The objective of the present study was to evaluate the antinociceptive, anti-inflammatory and antioxidant activities of dual extract of *P.nigrum* and *Anacyclus pyrethrum*. Extracts were taken by ultrasonification and found to have flavonoid, phenol, tannin and alkaloid. The extract of *P.nigrum* synergistically effective on DPPH at 1:0.5 ratio and vice versa exhibited 45% DPPH activity. The metal chelation of independent extract good at least concentration in both plants and better anti-inflammatory noted at *P.nigrum* extract at all tested concentration and moderate HRBC protection by *Anacyclus pyrethrum* shows effective at 25µg only. Like wise the extract of *P.nigrum* have promising antibacterial on *P.aeruginosa* and moderate activity given by *Anacyclus pyrethrum*.

ACKNOWLEDGEMENT

The authors acknowledgement Chancellor Shri.A.Srinivasan , Dhanalakshmi Srinivasan College of Institutions for the Financial support of this work.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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CITATION OF THIS ARTICLE

Suriya M., Surya Chinnasamy, Sujithra M., P.Shobana Devi and Swetza Singh: In vitro antioxidant, anti-inflammatory potential of *piper nigrum* fruit and *anacyclus pyrethrum* root extract. *Bull. Env.Pharmacol. Life Sci., Spl Issue* [5]: 2022: 697-705.