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Evaluation of antioxidant properties of *Strobilanthes Kunthiana* Nees t, Anderson ex, benth leaves organic solvent extracts

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Abstract
Plants are natural sources of antioxidants and recent research focuses on them for they are regarded as safe. Free radicals are causes of serious diseases because of the oxidative stress they cause on the body cells which leads to a series of conditions and diseases. There is a serious need of finding natural scavengers against the free radicals to combat the problem of free radicals. This study was carried out to evaluate the antioxidant activity of various extracts of *Strobilanthes kunthiana* leaves. Superoxide radical scavenging activity, Nitric oxide radical scavenging activity, Fe^{2+} chelating activity, ABTS radical decolorization assays were carried out. Ascorbic acid was used as standard. The half maximal inhibitory concentration values were determined in all the assays and was found to be lower in the ethanol extract; ABTS radical decolorization (60.05±0.04 µg/mL), $Fe2^{+}$ chelating (62.36±0.15 µg/mL), superoxide radical scavenging (IC_{50} = 62.24±0.26 µg/mL), and nitric oxide radical scavenging (66.27±0.52 µg/mL).Compared to all other extracts, ABTS radical decolorization assay showed better antioxidant activity as compared to other assays. In conclusion, the ethanol extract showed promising antioxidant activities and can be considered for active compound isolation and further analysis.

Keywords: antioxidant, *s. kunthiana*, organic solvent, extracts

1. Introduction
Antioxidant compounds are known to inhibit oxidation. Free radicals produced as a result of oxidation leads to a chain of reactions that damage cells of organisms causing oxidative stress. Oxidative stress balance in both plants and animals can be achieved by antioxidants like Vitamin C and Vitamin A among others. Antioxidants are found in vegetables and fruits and in some animal tissues such as lean meat and in some sea foods. Industrial use of antioxidants include acting as cosmetic and food preservative, and they also act as fuel oxidation inhibitors[1]. Free radicals which in turn cause oxidative stress leads to a number of pathological conditions such as aging, dementia, Parkinson's disease, arthritis, anaemia, ischemia, and diabetes [2, 3].

Plants are nature's secret cure to many ailments and are one of the most opted means of treatment since time immemorial. Plants have been used in part, synergistically to combat most of the complex ailments. Antioxidant based formulations have been used in diseases and conditions like diabetes, atherosclerosis and stroke [4]. Plants have been reported with various antioxidant compounds such as lignans, coumarins, flavons, alkaloids, flavonoids, catechins, tannins, and phenols [5, 6, 7]. The search for bioactive compounds from plants has continued and it adds value to the available knowledge because plants have many unique unexplored bioactive compounds [8]. Plants have also exhibited various bioactivities such as anti-inflammatory activities besides the antioxidant activities which gives as a strong foundation on the believe that plant compounds are safe [9, 10].

Strobilanthes kunthiana belongs to Acatheceae family which is reported for antioxidant activities [11, 12]. The plant is commonly found in the south India where it's famous for its long blooming gap. It's mainly used for ornamental purposes where it attracts a great number of tourists during its blooming seasons. There is little research on the pharmaceutical importance of these plant, a few studies that have been carried out on the plant shows that its ethanolic extract contains tannins, triterpenoids, steroids, flavonoids, phenols, glycosides, reducing sugars, alkaloids, and phytosterols. Flavonoids are well known to have antioxidant properties [13, 4]. In another study carried out by Isoe *et al* [14] *S. kunthiana* GC-MC revealed various bioactive compound including n-Hexadecanoic acid, 4-(3,5-Di-Tert-Butyl-4-Hydroxyphenyl, Squalene which have been known to contain antioxidant activities. With this basis this study was carried out to ascertain the facts. In nature mainly fruits and vegetables are renowned in terms of antioxidant potentials, little is going on, on wild plants like *S. kunthiana*. Therefore the following study was carried out to widen the knowledge on the bioactivities of *S. kunthiana.*

2. Materials and Methods
2.1 Plants sample collection
The leaves of *Strobilanthes Kunthiana* were collected from their natural habitat in Western Ghats Ooty, Tamil Nadu, India, and identified by Dr. S. Rajan field Botanist, the Survey of medicinal plants and collection unit, Government of India, Nilgiri.

2.2 Preparation of plant extraction
The leaves were washed thoroughly in tap water, shade dried at room temperature for 7 days and powdered using a grinder. Separately, 40 grams of powdered samples were dissolved in 400 ml of ethanol (1:10), Chloroform, ethyl acetate, methanol and petroleum ether and they were subjected to cold maceration for 48hours then kept on a rotary shaker at 190–220 rpm for 24 h. The extracts were

centrifuged (3000 X g) and clear supernatants were collected and filtered using Whatman No. 1 filter paper and evaporated dryness at 40°C by rotary evaporator (Buchi type, Flawil/Schweiz, Switzeland). The resulting crude extracts; ethanol (4g), Methanol (4g) ethyl acetate (3g), and petroleum ether (3g) were stored at 4 °C in airtight bottle for the analyses.

2.3 Fe^{2+} chelating activity assay

The chelating activity of leaves extracts petroleum ether, ethyl acetate, chloroform, methanol, and ethanol extract of *Strobilanthes Kunthiana* were evaluated by measuring the Fe^{2+} chelating activity according to the method of Rajic *et al*., [15]. Aliquote of 20- 100 µg/mL of the extract, 1.7 mL of distilled water and 0.05 mL of $FeCl_2$ (2 mM) were added and after 30 seconds, 0.1 Ml ferrozine (5 mM) were added [16]. The reaction mixture was incubated for 10 min at 30º C and the absorbance of the Fe^{2+} ferrozine complex was measured at 570 nm. A lower absorbance indicates a higher chelating power. The chelating activity of the extracts of Fe^{2+} was compared with that of EDTA (0.01 nM) and citric acid (0.025 M). The percentage of chelating activity calculated using the formula: % of chelating activity [17].

$$\text{Chalating activity (\%)} = \frac{\text{Control OD} - \text{Test OD X 100}}{\text{Control OD}}$$

2.4 ABTS. + Radical cation decolourisation assay

The leaves extracts of *Strobilanthes Kunthiana* were used and evaluated for their $ABTS^{\cdot +}$ Radical capacity followed Erel*et al*., [18]. method. The experiments were carried out using an improved ABTS decolourisation method. ABTS was generated by oxidation of ABTS with potassium persulfate [19]. Three mL of methanol extract solution in different concentration like 20-100 µg/mL. The decreasing in absorption was measured during 6 min at 570nm. The inhibition of the ABTS radical scavenging assay calculated using the above formula [20, 21].

2.5 Superoxide anion scavenging activity

The assessment of superoxide anion scavenging activity was done according to the method of Laight *et al*., [22]. The reaction mixture was of 20-100 µg/mL dilution of *Strobilanthes Kunthiana* leaves extract Petroleum ether, Ethyl acetate, chloroform, methanol, and ethanol and L ascorbic acid was made upto 1 mL with respective solvent. The 1 mL of phenazine methosulphate60 µM, in phosphate buffer 0.1M, pH 7.4 and 1 mL of nitro blue tetrazolium(NBT) (150 µM) in phosphate buffer 0.1M, pH 7.4. The reaction mixture was incubated at 25º c for 5 min, and the absorbance was measured at 570 nm [23]. The abilities to scavenge the superoxide radical and the percentage inhibition were calculated using the above formula [24].

2.6 Nitric oxide radical scavenging activity

Nitric oxide radical scavenging was carried out as per the method of Kumaran *et al*., [25]. Nitric oxide radicals were generated from sodium nitroprusside solution. 1 Ml of 10 mM sodium nitroprusside was mixed with 1 mL of 10 mM sodium nitroprusside was mixed with 1 mL of ethanol extracts of *Strobilanthes Kunthiana* leaves Petroleum ether, Ethyl acetate, chloroform, methanol, and ethanol aliquots of 20-100 µg/mL in phosphate buffer (0.2 M pH 7.4). The mixture was incubated at room temp for one hour. After incubation the reaction mixture mixed with 1.0 mL of pre prepared Griess reagent 1 % sulphanilamide, 0.1% napthylene diamine dichloride and 2% phosphoric acid [26, 27].The absorbance was measured at 570 nm and percentage of inhibition was calculated using formula above. The decreasing absorbance indicates a high nitric oxide scavenging activity [28].

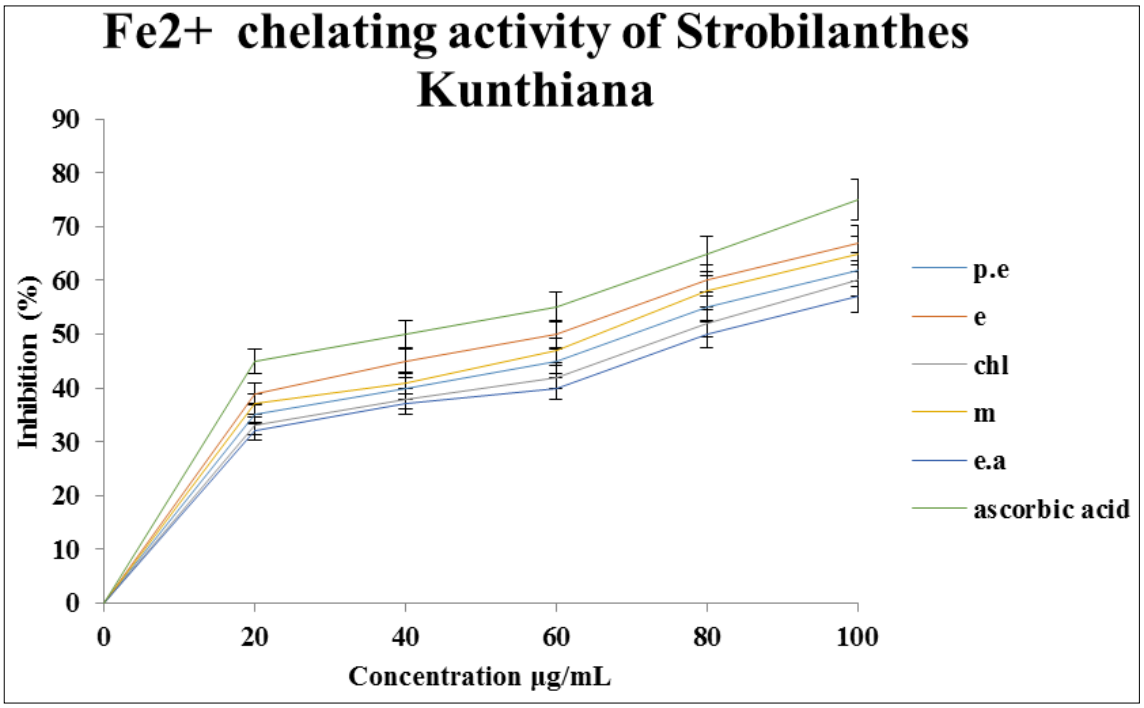
3. Results

3.1 Fe^{2+} chelating activity assay

Fe^{2+} chelating activity of *Strobilanthes Kunthiana* leaves extracts shows potent chelating power and the IC_{50} values of E 62.36±0.15 µg/mL, M 65.43±0.56 µg/mL, P.E 70.25±0.36 µg/mL, CHL 80.33±0.37 µg/mL, E.A 82.13±0.40 µg/mL and standard ascorbic acid found to be 42.27±0.24 µg/mL as shown in figure 1. The iron generates free radicals through the Fenton and Haber-Weiss reactions that prevent the oxidative damage.

Table 1: IC_{50} values of the *Strobilanthes Kunthiana* leaves extracts

S.NO	Organic solvents	*Strobilanthes Kunthiana* leaves extract (µg/mL)
1.	L ascorbic acid	42.27±0.24
2.	Petroleum ether	70.25±0.36
3.	Ethyl acetate	82.13±0.40
4.	Chloroform	80.33±0.37
5.	Methanol	65.43±0.56
6.	Ethanol	62.36±0.15



E-Ethanol, M- Methanol, CHL-Chloroform, E.A-Ethyl acetate, P.E-Petroleum ether

Fig 1: Fe^{2+} chelating activity of *Strobilanthes Kunthiana*

3.2 Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity of *Strobilanthes Kunthiana* leaves extracts performed by formation of nitric oxide using sodium nitroprusside. The sodium nitroprusside act as a major source of nitric oxide radicals. The extracts scavenges the nitric oxide formed from the sodium nitroprusside by inhibiting the chromophore formation. The inhibition was concentration dependent and it increased with increase in concentration. The IC_{50} values were E 66.27±0.52 µg/mL, M 74.27±0.71 µg/mL, P.E 78.40±0.38 µg/mL, CHL 83.37±0.41 µg/mL, E.A 88.21±0.62 µg/mL and that of L ascorbic acid was 50.36±0.12 µg/mL. The ethanol extract shows good scavenging ability compared to other solvent extracts (fig. 2).

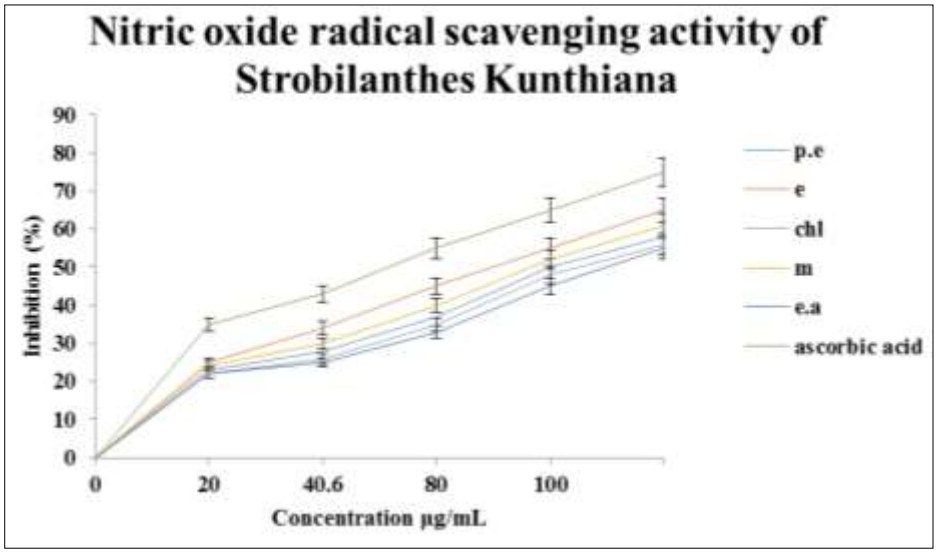


Fig 2: Nitric oxide radical scavenging activity of *Strobilanthes Kunthiana*

3.3 ABTS + radical cation decolourization assay

ABTS radical cation decolorization of *S. kunthiana* leave extracts are shown in figure 3, The IC_{50} values were; ascorbic acid (50.24±0.04 µg/mL), ethanol (60.05±0.04 µg/mL), methanol (64.26±0.07 µg/mL), petroleum ether (70.54±0.03 µg/mL), chloroform (78.48±0.0) and ethyl acetate (81.65±0.01 µg/mL). The potency of the different extracts is in the order in which they are presented, ethanol>methanol>petroleum ether>chloroform>ethyl acetate. These results show that there is some significant inhibition of the ABTS radical cation. The lower the $IC_{50,}$ the more effective the decolourization agent hence the ABTS radical scavenging ability found to be high in ethanol extract.

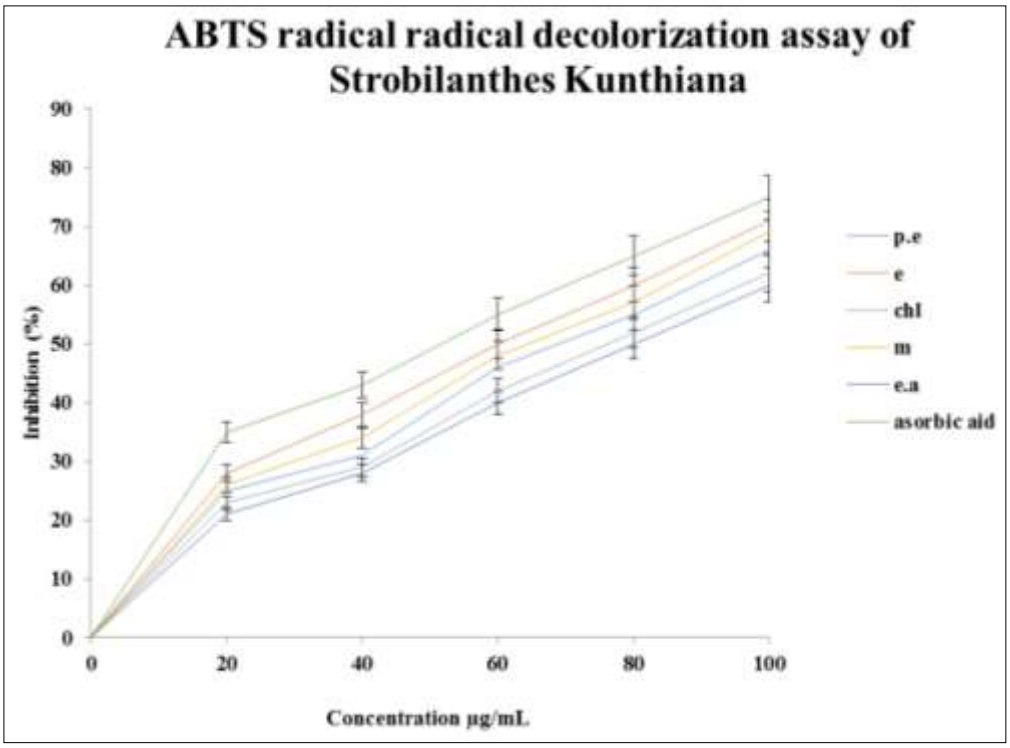


Fig 3: ABTS radical decolorization assay of *Strobilanthes Kunthiana*

3.4 Superoxide anion scavenging activity

The reduction of the yellow dye (NBT) to produce the blue formazan which was measured spectrophotometrically at 570 nm. The *Strobilanthes Kunthiana* L. leaves have potent antioxidants capacity. The decrease of absorbance at 570 nm indicates the high antioxidant power and the IC_{50} values of the *Strobilanthes Kunthiana* leave extracts was E 62.24±0.26 µg/mL, M 70.02±0.22 µg/mL, P.E 78.14±0.34 µg/mL, CHL 82.17±0.50 µg/mL, E.A 90.23±0.11 µg/mL and that of L ascorbic acid was 55.30±0.26 µg/mL respectively (Fig. 4).

Table 2: IC50 values of the *Strobilanthes Kunthiana* leaves extracts

S.No	Organic solvents	*Strobilanthes Kunthiana* leaves extract (µg/mL)
1.	L ascorbic acid	55.30±0.26
2.	Petroleum ether	78.14±0.34
3.	Ethyl acetate	90.23±0.11
4.	Chloroform	82.17±0.50
5.	Methanol	70.02±0.22
6.	**Ethanol**	**62.24±0.26**

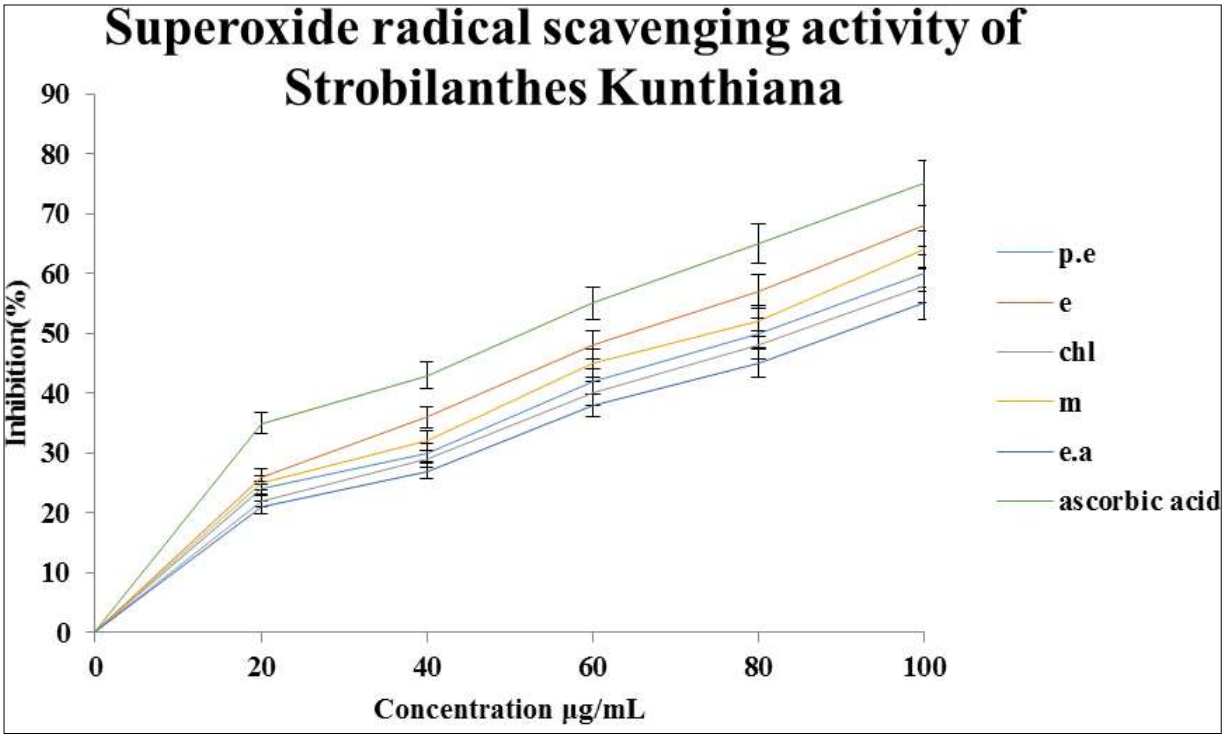


Fig 4: Superoxide radical scavenging activity of *Strobilanthes Kunthiana*

4. Discussion

Traditional plant based medicines also given a bulk practices across the country by placing them at the base of the medications. But such kind of knowledge is only embedded among the local people in the world. In such case we should have the ability to encourage such situations and thus cultivate the basics of the phytomedicines throughout the world. By considering this chance, the current working researchers in the world should have a best platform to cultivate the hidden knowledge behind the phytomedicines and their mode of action and curing capacity. The current research explains the Comparative evaluation of antioxidant properties in the *Strobilanthes Kunthiana* leaves extracts using organic solvents though various assays as a base to the drug discovery fields.

Iron is an essential mineral for normal physiological activity of the human body, but above the required limit may cause cellular injury and damage. The ferrous ions are more effective pro oxidants in food systems, the good chelating effects would be beneficial and removal of free iron ion from circulation could be a promising approach to prevent oxidative stress –induced disease [29]. The chelating ability of the extracts evaluated using the metal chelating with ferrozine on ferrous ion by forming a stable ion chelating. The high chelating power reduces the free ferrous ion concentration thus decreasing the Fenton reaction which is implicated in many diseases [30]. $ABTS^{\cdot+}$ Radical involves an electron transfer process [31].

The nitric oxides has key role in the human body specifically in numerous types of inflammatory processes, physiological process and it is also important as chemical mediator in endothelial cells, macrophages, neurons. The excess concentration of nitric oxide in human body may cause several diseases. The oxygen reacts with the excess nitric oxide to generate nitrate and petroxynitrite anions, which act as free radicals [32, 33].

The oxidative enzyme of a body produces superoxide from molecular oxygen through nonenzymatic reaction. The superoxide generate most hazardous oxygen species in human body such as singlet oxygen and hydroxyl radicals and these may cause the peroxidation of lipids. Superoxide anions are an ancestor of active free radicals and may react with biological macromolecules leading to tissue damage [34].

In a study conducted by Prabakaran and Kirutheka[12]where they used the methanol and ethanol callus extracts of *S. kunthiana*, their results showed higher antioxidant activity in methanol extract as compared to ethanol extract. In our study ethanol extract showed better activity than methanol extract, this could be due to the different plant samples used, they used callus unlike our study where we used the leave extract. The difference in their study could have also happened because they used different assays. It is also clear in their GC-MS results that the compound present in the callus extract are different from those reported in the leaf methanol extract [14].

Strobilanthes heyneana belonging to the same species is reported to contain, alkaloids, tannins, flavonoids and triterpenoids in its leaf extract which are reported in *S. kunthiana* too. These could be compounds that can be attributed to the bioactivities and there is need to isolate and know which of these compounds are specifically having the antioxidant components of the crude extracts [11, 35]. The study showed a dose dependent inhibition which is in line with our study and ethanol extract was shown to have positive bioactivities. Other Strobilanthes species have been reported to possess ferric reducing efficacies. Ethanol has also been used as one of the most suitable solvents of choice in the Strobilanthes species studies[36, 37, 38].

4. Conclusion

In our research, the ethanol extract of *Strobilanthes Kunthiana* leaves extracts was found to be effective compared to other organic solvents hence preferred solvent for further isolation of molecule from the *Strobilanthes Kunthiana* leaves extracts.

5. Acknowledgement

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6. Conflict of Interest

Authors declare no conflict of interest

7. References

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