How to Cite:
Surwase, U. S., & Patil, S. M. (2022). Antioxidant and antipyretic studies on methanolic extraction of flaveria trinervia leaves. *International Journal of Health Sciences*, *6*(S3), 9803–9818. https://doi.org/10.53730/ijhs.v6nS3.9477

Antioxidant and antipyretic studies on methanolic extraction of flaveria trinervia leaves

Ulhas Shrimant Surwase
K.T. Patil College of Pharmacy. Osmanabad, 413501, Maharashtra, India
*Corresponding author email: ulhaspharmacy22@gmail.com

Shivanand Malkappa Patil
Shree Dev Bhoomi Institute of Education. Science & Technology Department of Pharmaceutical Science, Poundha, 248007, Dehradun, Uttarakhand, India

***Abstract*---**The aim of the present study was to perform antipyretic and antioxidant activity of Flaveria trinervia Methanolic leaves extract by different in-vivo and in-vitro models. The different pharmacognostical parameters were evaluated as per standard procedure. Finally, antipyretic (Brewer's Yeast Pyrexia model) and antioxidant activity (Hydroxyl Radaical scavenging activity, determination of Reducing Power, Metal chelating activity, Carbon tetrachloride (ccl4) induced lipid peroxidation, Inhibitory Test on Protein Oxidative Modification, Lipid peroxidation assay, Catalase assay and Reduced glutathione assay) was evaluated by adopting different methods. The extracts showed a marked antipyretic effect by causing a reduction in yeast-induced fever. Methanolic extract (200 and 400 mg/kg) showed the effect to the same degree as paracetamol (50mg/kg, i.p.). The experimentally induced laboratory model was employed in evaluating the antipyretic activities of Methanolic extracts of Flaveria trinervia. The extract caused a better hypothermal activity against yeast-induced pyrexia in rats. Free radical scavenging activity of Flaveria trinervia. was found because polyphenolic compounds present in plant contribute significantly to the total antioxidant capacity of the leaves. Flavonoids play some important pharmacological roles against diseases, such as cardiovascular diseases, cancer, inflammation and allergy. The results of the study indicate that the Methanolic leaves extract of Flaveria trinervia possesses strong antipyretic and antioxidant activity. This study described many pharmacognostical features and antioxidant activity of leaves of Flaveria trinervia which will give a new direction for the future scientific research.

Keywords---flaveria trinervia, hydroxyl radaical scavenging, catalase assay, reduced glutathione assay.

Introduction

Herbal medicines are assumed to be of great importance in the primary healthcare of individuals and communities in many developing countries (Ghosh A, 2003). For thousands of years, these natural plant products have been utilized for human healthcare in the form of drugs, antioxidants, flavours, fragrances, dyes, insecticides and pheromones. However, during the last century the use of synthetic drugs led to a decline in the use of plant-derived compounds, so that the synthetic drugs would perhaps completely replace the use of traditional plant-derived medicines (Gomez-Galera S et al., 2000). Naturally, there is a dynamic balance between the amount of free-radicals generated in the body and antioxidants to quench and/or scavenge them and protect the body against their deleterious effects (Nose K, 2000). Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and our metabolism. They are continuously produced by the body's normal use of oxygen such as respiration and some cell-mediated immune functions. They are also found or generated through environmental pollutants, cigarette smoke, automobile exhaust fumes, radiation, air pollutants, pesticides etc. However, the amount of these protective antioxidant principles present under the normal physiological conditions, are sufficient only to cope with the physiological rate of free-radical generation.

Therefore, it is obvious that any additional burden of free-radicals either from environment or produced within the body can alter the pro-oxidant and antioxidant balance leading to oxidative stress. Reactive oxygen species (ROS) is a collective term for oxygen-centered radicals such as superoxide, hydroxyl and non- radical oxygen derivatives, namely hydrogen peroxide and singlet oxygen (Suzuki N and Mittler R, 2006). In humans the over production of ROS can result in tissue injury and has been implicated in disease progression and oxidative damage of nucleic acids and proteins (Middleton JRE et al., 2000). When there is a lack of antioxidants to quench the excess reactive free radicals, cardiovascular, cancer, neurodegenerative, Alzheimer's and inflammatory diseases may develop in the body (Krishnaiah D et al., 2010). Thus the antioxidant status in human reflects the dynamic balance between the antioxidant defense and pro-oxidant conditions and has been suggested as a useful tool in estimating the risk of oxidative damage. Due to the benefits of antioxidants, food and pharmaceutical products are normally supplemented with synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butyl hydroxyquinine (TBHQ). However, the dietary intake of synthetic antioxidants could cause genotoxicity and carcinogenicity at high concentrations (Gutteridge JMC and Halliwell B, 2010). In addition, natural antioxidants from plant products may be more effective in reducing ROS levels compared to synthetic single dietary antioxidants due to the synergistic actions of a wide range of biomolecules such as vitamins C and E, phenolic compounds, carotenoids, terpenoids and phytomicronutrients (Perez-Jimenez J et al., 2008).

Antipyretics are drugs that reduce body temperature in situations such as fever which is a part of the body's immune response to infection. However, they will not affect the normal body temperature if one does not have a fever. A rise in blood temperature leads to manifestations of heat loss which increases heat production. Antipyretics cause the hypothalamus to override an interleukin-induced increase in temperature. The body will then work to lower the temperature and the result is a reduction in fever. The non-steroidal anti-inflammatory drugs are antipyretic, anti-i inflammatory, and pain relievers (Keele CA, 1969). Search for herbal remedies with potent antipyretic activity received momentum recently as the available antipyretics, such as paracetamol, nimusulide etc. have toxic effect to the various organs of the body (Guyton AC and Hall JE, 1998).

Flaveria trinervia is a species of flowering plant in the aster family known by the common names clustered yellowtops, speedyweed, and yellow twinstem. It is native to parts of the Americas, including the southeastern and southwestern United States (Florida, Texas, Arizona, New Mexico), most of the Bahamas, Mexico, Belize, and parts of the Caribbean, especially Cuba, Cayman Islands, Jamaica, Hispaniola, Puerto Rico and Barbados. It is also known in many other places as an introduced species and often a noxious weed, such as in Hawaii (Hawaii Powell and Albert Michael 1979). The present study was to evaluate the antioxidant and antipyretic studies on Methanolic extraction of *flaveria trinervia* leaves.

Materials and Methods

Plant collection and authentication

Leaves of *flaveria trinervia* were obtained from the local places of Tirupati, AP. *flaveria trinervia* Plant was authenticated by Dr. K. Madhava Chetty, M.Sc., M.Ed., M.Phil., Ph.D., PG DPD., Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, and Andhra Pradesh.

Extraction by Maceration

Fresh leaves were washed with water to get rid of contaminants like dirt and other impurities and were shade-dried. These dried leaves were ground and sieved to get a uniform, coarse powder. Powdered plant material was weighed (1Kg) and is immersed in Methanol and kept for maceration for a period of 7 days with occasional stirring. On the 8^{th} day, the solvent was filtered by pressing with a muslin cloth and was evaporated in a rotary evaporator at $40^{o}C$. The resultant extract was put in a desiccator to remove any ethanol left in it. The dried Methanolic extract of *Flaveria Trinervia*. (MEFT) was packed in an air-tight bottle and put in a dry place for further studies [Saxena K, 2016, Bint-e-Sadek Y et al., 2013]

Qualitative evaluation of phytoconstituents

The MEFT was screened for the presence of various phytoconstituents like carbohydrates, flavonoids, polyphenolic compounds, saponins, tannins, triterpenoids, etc.

***In vitro* antioxidant studies**
Hydroxyl Radaical scavenging activity

The hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+} / ascorbate / EDTA / H_2O_2 system. The hydroxyl radicals attack deoxyribose, which eventually results in TBARS formation. The reaction mixture contained deoxyribose (2.8mM), $Fecl_3$ (0.1mM), H_2O_2 (1mm), ascorbate (0.1mM), KH_2PO_4-KOH buffer (20mM, pH 7.4) and various concentrations (MEFT 100, 200, and 300 µg/ml and standard Mannitol 100µg/ml) of the drug in a final volume of 1 ml. The reaction mixture was incubated for 1 hr at 37°C. Deoxyribose degradation was measured at 532nm (Mary NK et al., 2002).

Determination of Reducing Power

The reducing power of MEFT was determined according to the following method. Various concentrations (125,250,175 and 500µg/ml) of extract of MEFT in 1 ml of distilled water was mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and potassium ferricyanide (K_3Fe (CN) $_6$) (2.5ml, 1%).The mixture was incubated at 50°C for 20 min. A portion (2.5ml) of trichloroacetic acid (15%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and ferric chloride (0.5ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power (Gulcin I et al., 2002).

Metal chelating activity

Metal chelation property for Ferric ion (Fe^{3+}) was estimated by using thiocynate method. Here different ratio of the extract (1:0.25 to 1:10 ratio) was mixed with a fixed concentration of ferric chloride (10µg). The mixture was incubated for 30 min. At the end of the incubation, 1ml of potassium thiocyanate (25%) was added and absorbance of ferric-thiocyanate complex (reddish brown complex) was measured at 460 nm. The results were compared with EDTA (1:10). Metal chelation property for ferrous ion (Fe^{2+}) was estimated by using 2, 2-bipyridyl method. Here different concentrations of the extract were mixed with a fixed concentration of ferrous sulphate (10µg). The mixture was incubated for 30 min. At the end of the incubation, 2ml of 2, 2-bipyridyl (1mM) was added and absorbance of ferrous –bipyridyl complex (pink-colored complex) was measured at 525 nm. The results were compared with EDTA (Tripati YB et al., 2001).

Carbon tetrachloride (ccl_4) induced lipid peroxidation

Rat liver (30%w/v) homogenate in ice – cold 0.15 M potassium chloride was prepared in homogenizer. Aliquots of 0.5 ml of homogenate were taken in different small conical flasks. These flasks were incubated at 37°C in a constant shaker bath (150 cycles/min) for 45 min with 1.5 ml of potassium phosphate buffer (pH 7.4), 2ml of 0.15 M potassium chloride, MEFT at (25,50,100,200 and 300 µg/ml) and Vitamin – E 100 µg/ml in different flasks and finally 10 µl of carbon tetrachloride (CCl_4) was added. In case of control, both CCl_4 and drugs were not

added and in some flasks only drug was excluded. The reaction was stopped by the addition of 4 ml of 10%(w/v) tri chloro acetic acid and after incubation, the contents were centrifuged at 4000 rpm for 10 min and about 2ml of clear supernatant was transferred to a graduated tube and 2 ml of 0.67%w/v of thiobarbituric acid was added and heated in a boiling water bath for 15 min. The tubes were cooled, bringing the mixture to pH 12-12.5 with potassium hydroxide, stabilized the colour developed, and the absorbency was measured at 543nm (Comporti M, 1989).

Inhibitory Test on Protein Oxidative Modification

Albumin oxidative modification by copper was performed by the following method. The test sample (MEFT 100-1000 µg/ml) and Vitamin- E (100-1000µg/mi), was added to the reaction mixture containing albumin (10µg/ml) and 100µM CuCl2 in 50mM Tris- HCL buffer (pH 7.4) in a total volume 0.3ml. The mixture was incubated at 37^{o}C for 2 hr. Next 1.6ml of 0.125 M phosphate buffer (P^{H} 8.0) containing 12.5mM EDTA and 10.0 M urea, and 0.1 ml of 50 mm phosphate buffer (P^{H} 7.0) containing 10mM DTNB were added to the reaction mixture. This solution was allowed to stand at room temperature for 5 min. The absorbency was read at 412 nm as cysteine-SH residue (Toda S et al., 1999).

***In vivo* anti-oxidant studies**
Animal Studies
Toxicity studies

Albino rats (200-200gm) of either sex were selected and segregated in to 8 groups of 6 animals each. Single dose of Methanolic extract of *Flaveria Trinervia*, starting from the minimal dose of 50mg/kg up to 3000mg/kg administered orally. The drug treated animals were observed carefully for its toxicity signs and mortality. From the maximum dose, 1/5th and 1/10th of the concentration was considered as therapeutic dose for further study.

Animals

Albino rats (175-225gm) of either sex and of approximate same age used in the present studies were procured from Central Animal facility, CMR college of Pharmacy, Hyderabad, India. The animal was fed with standard pellet diet and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours in darkness and light. The animals were acclimatized to the laboratory condition for a one week before starting the experiment. The experiment protocols were approved by Institutional Animal Ethics committee after securitization (IAEC No: CPCSEA/1657/IAEC/CMRCP/COL-19/67). The animal received the drug treatment by oral gavage tube.

Animal treatment

Twenty-four male albino rats (n = 6) were divided into four different groups. Group I served as a control group and treated with vehicle only (0.5% carboxymethylcellulose sodium). Group II served as disease control, Group III

served as standard control Group IV and V animals were administered orally with 250 and 500 mg/kg of MEFT, respectively, for 7 days. At the end of 7th day, the animals were sacrificed by cervical dislocation and each brain was excised, rinsed in ice-cold normal saline and followed by 0.15 M Tris-hydrochloride. The homogenates were centrifuged at 15,000 × g for 10 min. The supernatants were employed for the following assays.

Lipid peroxidation assay (thiobarbituric acid reactive substances)

It was evaluated by thiobarbituric acid reactive substances (TBARS) tests during an acid-heating reaction. Aliquots of samples were incubated with 15% trichloroacetic acid and 0.38% thiobarbituric acid. The mixture was heated (1 h) in a boiling water bath. TBARS was determined by reading the absorbance of the pink-colored complex formed in a spectrophotometer at 532 nm (Satoh K, 1978).

Catalase assay

It was determined with reaction solution contained 2.5mL of 0.05 M phosphate buffers (pH 8.3), 0.7 mL of 0.2 M H_2O_2 and 0.1mL of tissue homogenate. Changes in absorbance of the reaction solution at 570 nm were determined after 1 min. Results were expressed in units/mg protein (Maehly AC and Chance B. I, 1954).

Reduced glutathione assay

This was estimated by using dithiobisnitro-benzoate as a substrate. The yellow color developed and read immediately at an absorbance of 412 nm and expressed as μM GSH/g protein.

Statistical analysis

The values were expressed in mean ± standard error of the mean. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison tests versus control. $P < 0.05$ and $P < 0.01$ were considered as significant (Ellman GL, 1959).

Antipyretic Activity

Yeast induced pyrexia was used to evaluate the antipyretic activity of the test compounds. The body temperature of each rat was recorded by measuring the rectal temperature at predetermined time intervals. Fever was induced by injecting 15% suspension of Brewer's yeast (*Saccharomyces cerevisiae*) following a standard method. The rats were allowed to remain quiet in the cage for some time. A thermister probe was inserted 3-4cm deep into the rectum after fastening the tail to record the basal rectal temperature. The animals were then given a subcutaneous injection of 10 ml/kg of 15%w/v Brewer's yeast suspended in 0.5% w/v CMC solution and the animals were returned to their housing cages. Nineteen hours after yeast injection, the rats were again restrained in individual cages to record their rectal temperature. Immediately the test compounds and standard were administered orally at their respective doses. Rectal temperature of all the rats was recorded at 19 h immediately before the administration of test

compounds, vehicle and paracetamol (50 mg/kg.) and again at 1hour intervals up to 3hours after the administration (Vogel HG, 2002).

Table 1
Results of Phytochemical screening

S. No	Name of the Phytochemical	MEFT
1.	Carbohydrates	+
2.	Amino acids	+
3.	Proteins	+
4.	Alkaloids	+
5.	Cardiac glycosides	+
6.	Triterpenoids	+
7.	Saponins	+
8.	Flavonoids	+
9.	Phenolic compounds	+
10.	Tannins	+
11.	Steroids	-
12.	Gums	-

Where, + means positive and - means negative.
The preliminary phytochemical screening showed the presence of various phytoconstituents like flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, and carbohydrates in MEFT.

***In Vivo* Antioxidant Studies**

Table 3
Effect of MEFT on LPO, GSH, and CAT

S.No	Treatment groups	Lipid peroxidation (in µM/mg tissue)	Reduced glutathione (in µM of GSH/mg tissue)	Catalase (in units/mg protein)
1.	Normal control	2.85±0.08	4.22±0.12	0.720±0.04
2.	Disease control	5.23±0.21	2.90±0.13	0.450±0.03
3.	Standard control	2.96±0.16***	3.61±0.08***	0.637±0.02**
4.	MEFT 200mg/Kg	3.45±0.19***	3.47±0.57***	0.578±0.07*
5.	MEFT 400mg/Kg	3.67±0.65***	3.68±0.68**	0.613±0.03*

Values are represented as Mean ± SEM. Statistical analysis was done by one way ANOVA followed by post hoc Dunnett's multiple comparison tests. $^{***}p<0.0001$, $^{**}p<0.001$, and $^{*}p<0.05$ vs Disease control.

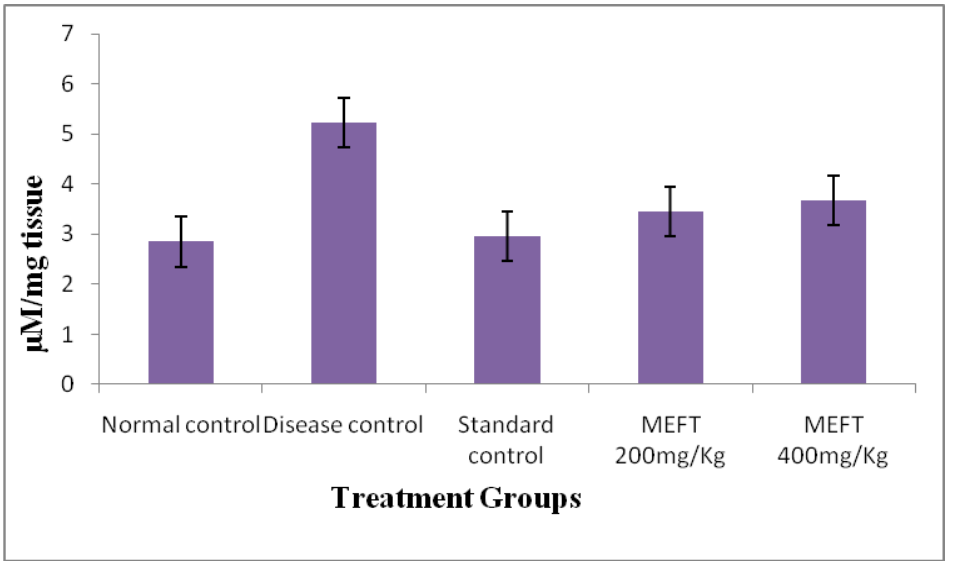

Figure 1. Effect of MEFT on LPO levels

The significant values of LPO levels of normal, disease, standard, MEFT 100mg/Kg and MEFT 200mg/Kg were found to be 2.85±0.08, 5.23±0.21, 2.96±0.16, 3.45±0.19, and 3.67±0.65 respectively on Day 29. There is a significant decrease in LPO levels of animals treated with MEFT 200mg/Kg and 400mg/Kg compared to disease control.

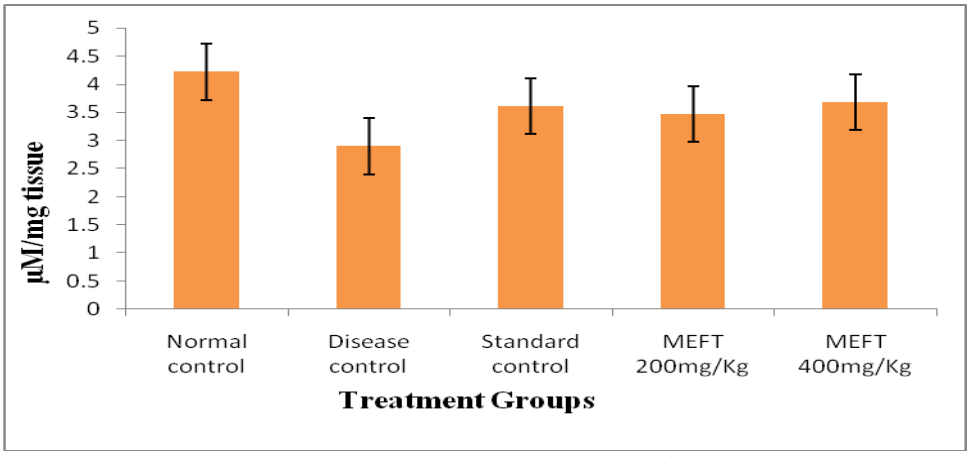

Figure 2. Effect of MEFT on GSH levels

The significant values of GSH levels of normal, disease, standard, MEFT 100mg/Kg and MEFT 200mg/Kg were found to be 4.22±0.12, 2.90±0.13, 3.61±0.08, 3.47±0.57 and 3.68±0.68 respectively on Day 29. There is a significant increase in GSH levels of animals treated with MEFT 200mg/Kg and 400mg/Kg compared to disease control.

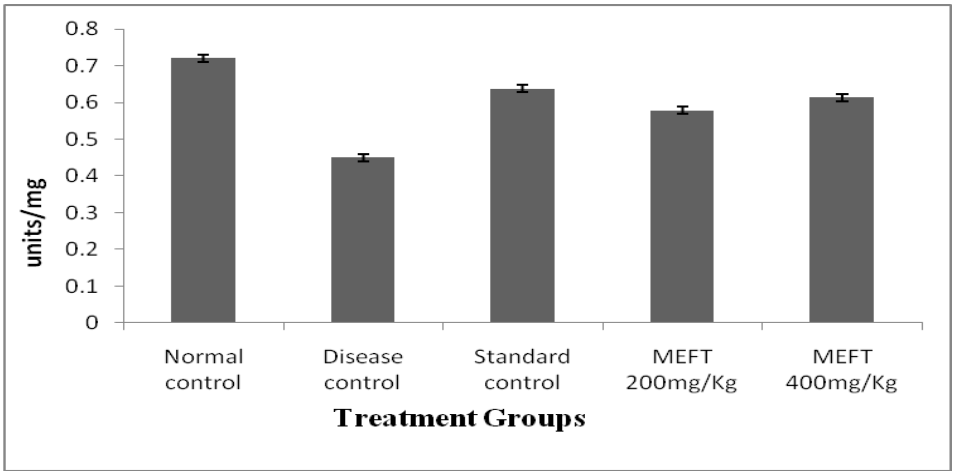

Figure 3. Effect of MEFT on CAT levels

The significant values of CAT levels of normal, disease, standard, MEFT 100mg/Kg and MEFT 200mg/Kg were found to be 0.72±0.04, 0.45±0.03, 0.63±0.02, 0.578±0.07, and 0.613±0.03 respectively on Day 29. There is a significant increase in CAT levels of animals treated with MEFT 200mg/Kg and 400mg/Kg compared to disease control.

***In-vitro* anti-oxidant study**

Hydroxyl radical scavenging activity

The MEFT (at all tested doses 100µg, 200µg and 300µg) significantly (P<0.001) scavenged the hydroxyl radicals generated by the EDTA/H_2O_2) system, when compared with that of control. The percentage scavenging of OH radicals by MEFT increased in a dose depended manner. Results were comparable standard (Mannitol100ug), (P<0.001). Results were shown in Table 4.

Determination of reducing power

The reducing power of MEFT increased with increasing concentration of MEFT. All the tested concentrations of MEFT showed significant (P< 0.001) activity than control. Results were comparable with the standard (BHT) (P<0.001). Results were shown in Table 5.

Effect of MEFT on fe^{2+} and fe^{3+} metal chelation

MEFT chelated Fe^{2+} (65.18±9.43%) and Fe^{3+} (55.19±8.54) significantly (P<0.001) at 1:10 ratio of iron : MEFT and chelating ability for mental transition ions (Fe^{2+},Fe^{3+}) increased in a dose dependent manner respectively. MEFT at all tested concentrations exhibited significant (P<0.001) chelation, when compared against control. In similar conditions, EDTA exhibited 78.64% chelation for $Fe^{2+}$and 85.42% for Fe^{3+} respectively, which is significant (P<0.001) when compared with the control. Results were shown in Table 6.

Lipid Peroxidation Induced By CCl_4

Lipid peroxide formation from CCl_4 was significantly ($P<0.001$) inhibited by MEFT at all tested dose levels (25µg, 50µg, 100µg, 200µg and 300µg) when compared with that of control. The percentage inhibitions of peroxide formation increased in a dose dependent manner. Results were comparable with that of standard. Results were shown in Table 7.

Inhibitory test on protein oxidative modification

The inhibitory ratio of MEFT on albumin oxidative modification was as high as 78.94 at a concentration of 1000µg/ml and increased in a concentration dependent manner. The EC50 of MEFT was found to be 427.86±7.351µg/ml. The results were comparable with the standard (Mannitol), with percentage inhibitory ratio of 81.99% at a concentration of 1000µg/ml. The IC_{50} of Mannitol was found to be 263.35 ±7.41 µg/ml. Results were shown in Table 8.

Table 4
Hydroxyl radical scavenging activity MEFT and Mannitol

S. No	Concentration (µg/ml)	% Inhibition Of Hydroxyl Radical
1.	Control	-
2.	MEFT (200)	76.3±12.12*
3.	MEFT (400)	81.33±13.16*
4.	Standard (Mannitol 100µg)	81.62±12.177*

Statistical significant test for comparison was done by ANOVA, followed by Dunnet's "t' test, *p< 0.001, when test and standard are compared against control, Values are Mean± SEM.

Table 5
Determination of reducing power of MEFT and BHT

S. No	Concentration (µg/ml)	Absorbance (OD)
1	Control	0.086± 0.000392
2	MEFT (400)	1.0122±0.00042*
3	MEFT (375)	0.9632±0.00054*
4	MEFT (200)	0.5431±0.00067*
5	MEFT (125)	0.3432±0.00071*
6	BHT(400)	0.6288±0.00070*
7	BHT(375)	0.4935±0.0037*
8	BHT(200)	0.389±0.00073*
9	BHT(125)	0.300±0.00110*

Statistical significant test for comparison was done by ANOVA, followed by Dunnet's't' test
*p< 0.001, when compared against control. Spectrophotometric deduction of the Fe^{3+} - Fe^{2+} transformation. Values are Mean± SEM

Table 6
Effect of MEFT and EDTA on Fe^{2+}/ Fe^{3+} metal chelation

Iron : Drug	OD at 525 nm	% Chelation of Fe^{2+}	OD at 460nm	% Chelation of Fe^{3+}
1:00(control)	0.308	0	1.043	0
1:0.25 MEFT	0.232	24.23±3.19*	0.853	13.44±2.93*
1:0.5 MEFT	0.218	32.69±2.35*	0.767	22.33±2.17*
1:1 MEFT	0.211	35.45±7.25*	0.787	29.94±2.46*
1:2.5 MEFT	0.198	42.84±8.26*	0.646	31.78±6.43*
1:5 MEFT	0.173	53.25±7.17*	0.674	36.43±5.43*
1:10 MEFT	0.136	65.18±9.43*	0.436	55.19±8.54*
(1:10)Standard (EDTA)	0.067	78.64±10.204*	0.149	85.42±8.36*

Statistical significant test for comparison was done by ANOVA, followed by Dunnet 's 't' test, Fe^{2+} and Fe^{3+} were quantitated by Fe2+ -dipyridyl complex (525 nm) and
Fe^{3+} - thiocynate complex (460nm), respectively. *p< 0.001, when test and standard are compared against control, EDTA: Ethylene diamine tetra acetic acid

Table 7
Inhibition of lipid peroxidation –induction by CCl_4 system of MEFT and Vitamin-E

S. No	Concentration (µg/ml)	% Inhibition
1	Control	-
2	MEFT (25)	21.59±2.43*
3	MEFT (50)	38.15±3.35*
4	MEFT (100)	42.99±9.25*
5	MEFT (200)	51.37±7.32*
6	MEFT (300)	60.90±8.65*
7	Standard (Vitamin- E)	66.27±7.25*

Statistical significant test for comparison was done by ANOVA, followed by Dunnet 's 't' test (n=6), *p< 0.001, when test and standard are compared against control, Values are Mean± SEM.

Table 8
Inhibitory test on protein oxidative modification of MEFT and Vitamin-E

S. No	Concentration (µg/ml)	% inhibition	IC_{50} Value (µg/ml)
1	MEFT(100)	23.47±2.38	
2	MEFT (200)	44.35±3.23	
3	MEFT (400)	57.26±2.17	
4	MEFT (600)	66.12±3.24	427.86±7.351
5	MEFT (800)	72.95±5.16	
6	MEFT (1000)	80.96±3.13	
7	Standard (Vitamin-E100)	32.15±0.079	
8	Vitamin-E(200)	51.68±0.242	

9	Vitamin-E(400)	63.22±0.042	
10	Vitamin-E(600)	72.18±0.052	263.35±7.47
11	Vitamin-E(800)	80.26±0.106	
12	Vitamin-E(1000)	81.99±0.055	

Values are Mean± SEM.

Table 2
Antipyretic activity effect of MEFT on yeast induced pyrexia

Treatment Groups Dose (mg/kg)	Yeast induced Pyrexia (Temp. in °C) 0h	1/2h	1h	3h
Control	37.66±0.26	37.48±0.22	37.24±0.15	36.66±0.18
Paracetamol (50 mg/kg.)	37.46±0.12	36.94±0.06*	36.66±0.07*	35.44±0.11**
MEFT 200mg	35.72±2.18	35.54±3.29**	35.42±4.38**	35.32±4.34**
MEFT 400mg	37.14±5.13	36.54±5.16*	36.67±5.15**	36.45±3.66

Data presented as mean ± S.E.M. n=6, *** *P*< 0.001, ** *P*< 0.01, * *P*< 0.05, Compared with control group, followed by ANOVA followed by Post hoc test (Dunnett's't' test)

Discussion

The presence of carbohydrates, proteins and amino acids in all the parts of the plant reveals that it can be used as a good nutritional supplement, if it is free from antinutritional factors. It is also noted that the other phytochemical compounds detected are known to have beneficial use in pharmaceutical industries. The higher amount of alkaloids present in all the parts of the plant can make the plant to be a good source of alkaloids which can be isolated and then purified. In medicine, saponin is used in hypercholestrolaemia, hyperglycaemia and as antioxidant, anticancer, anti-inflammatory etc (Ngbede J et a., 2008). Therefore the presence of higher amount of saponins in leaves of *Flaveria trinervia* makes t a good source for both industrial and medical purposes. Tannins were reported to exhibit antiviral, antibacterial and antitumour activities. It was also reported that certain tannins are able to inhibit HIV replication selectively and is also used as a diuretic. Since all the parts of plant contain total tannin in appreciable amounts, it is clear that *Flaveria trinervia* can have the ability to cure such viral and bacterial diseases. The phenolics and flavonoids have got much attention in the day to day life due to their antimutagenic, antitumour and antioxidant activities. Therefore, the estimation and characterization of stem and root extracts of *Flaveria trinervia* for phenolics and flavonoids should be done to explore bioactive principles of such compounds.

Metal chelating capacity was significant as they reduced the concentration of the catalyzing transition metal in lipid peroxidation (Duh PD et al., 1999). Through epidemiological studies, it was reported that phenolic compounds have been shown to act as natural antioxidants by helping to neutralize free radicals and as metal chelating agents (Ali SS et al., 2008). Antioxidants inhibit interaction between metal and lipid through formation of insoluble metal complexes with

ferrous ion. Hence the data obtained for *Flaveria trinervia* reveals that some of the extracts demonstrate an effective capacity for iron binding, suggesting that its action as antioxidant may be related to its iron binding capacity that will prevent the free radical generation through Fenton reaction.

Superoxide radical is known to be a very harmful species to cellular components as a precursor of more reactive oxygen species. The superoxide radical is known to be produced invivo and can result in the formation of H_2O_2 via dismutation reaction. Moreover, the conversion of superoxide and H_2O_2 into more reactive species, eg., the hydroxyl radical, has been thought to be one of the unfavourable effects caused by superoxide radicals (Halliwell B, 1991). Numerous biological reactions generate superoxide radical which is a highly toxic species. Although they cannot directly initiate lipid oxidation, superoxide radical anions are potent precursors of highly reactive species such as hydroxyl radical and thus the study of scavenging of this radical is important (Kannat SR et al., 2007). Since *Flaveria trinervia* showed appreciable percentage of scavenging activity for hydrogen peroxide, it can be used against unfavourable effects caused in the body by hydrogen.

Hydrogen peroxide itself is not very reactive but sometimes it is toxic to cell because it may give rise to hydroxyl and peroxyl radicals in the cells through Fenton reaction. Therefore, removing of H_2O_2 is very important for antioxidant defence in cell or food systems. Dietary polyphenols have also been shown to protect mammalian and bacterial cells from cytotoxicity induced by hydrogen peroxide, especially compounds with the orthodihydroxy phenolic structure, quercetin, catechin, gallic acid ester and caffeic acid ester [Nakayama T, 1994, Okafor OY et al., 2011, and Chakraborty SP et al., 2011]. Therefore, the methanol extract of *Flaveria trinervia* can be used as a potent hydrogen peroxide scavenger in body systems Nitric oxide or reactive nitrogen species, formed during their reaction with oxygen or with superoxides, such as NO_2 , N 2 O 4 , N 3 O 4 , NO 3 - and NO 2+ are very reactive. These compounds are responsible for altering the structural and functional behavior of many cellular components. The plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to the human health. Nitric acid is also implicated for inflammation, cancer, and other pathological conditions (Moncada A, 1991). Since the Methanolic extracts of *Flaveria trinervia* showed good activity it is clear that it can be used for scavenging reactive nitrogen species in human body.

Fever may be due to infection or one of the sequels of tissue damage, graft rejection and/or other disease states. Antipyretics are the agents which reduce the elevated body temperature. Yeast-induced pyrexia is called pathogenic fever and its etiology involves production of prostaglandins, which set the thermoregulatory centre at a lower temperature. The production of prostaglandins, mainly the most potent pyretic agent, PGE_2 appears to be a final pathway responsible for fever production induced by several pyrogens. The antipyretic activity is generally exhibited as one of the properties of non steroidal anti-inflammatory drugs, resulting from their inhibitory effect on prostaglandin

biosynthesis in the central nervous system [Howard M, 1993, Singh R.K and Acharya S.B 2000] Methanolic extract of *flaveria trinervia* dose dependently exhibited significant ($p < 0.05$) antipyretic activity in yeast-induced elevation in body temperature in rats and the effects are comparable to the reference antipyretic drug (paracetamol). It appears that the observed antipyretic activity of *flaveria trinervia* may be due to inhibition of prostaglandin synthesis. Again the extracts contain flavonoids and saponins, the antipyretic potential of which have been reported in various studies (Martinez-Vazquez M et al., 1996, Reanmongkol W et al., 2007). Therefore, the activity may be due to presence of the above group of phytoconstituents in *Flaveria Trinervia.*

Conclusion

Antioxidants are important in the prevention of human diseases. Compounds with antioxidants activity may function as free radical scavengers, complexers of pro-oxidants metals, reducing agents, and quenchers of single-oxygen formation or reactive oxygen species, thereby protecting the body from degenerative diseases such as cancer. The reactive oxygen species (ROS) are harmful byproducts generated during normal cellular metabolism or from toxic insult. They lead to a state of oxidative stress that contributes to the pathogenesis of a number of human diseases by damaging lipids, proteins and DNA. This has inspired much interest in antioxidant activity of phytochemicals. In this study, the choice of ethanol as an extractant was because of its availability and affordability at the time of this study. Our results have shown ethanol extracts from *flaveria trinervia* displayed strong antioxidant and antipyretic activity.

References

Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahu A (2008). Indian medicinal herbs as sources of antioxidants. Food ResInt; 41: 1-15.

Bint-e-Sadek Y, Choudhury N, Shahriar M (2013). Biological investigations of the leaf extracts of Carissa Carandas. International Journal of Pharmaceutical Research and Technology. 5:97-105.

Chakraborty SP, Mahapatra SK, Kumar SS, Pramanik P, Roy S (2011). Antioxidative effect of folate-modified chitosan nanoparticles. Asian Pac J Trop Biomed; 1(1): 29-38.

Comporti M (1989). Three models of free radical-induced cell injury. 72:1-56.

Duh PD, Tu YY, Yen GC (1999). Antioxidant activity of water extract of Harng Jyur Chrysanthemum morifolium Ramat. Lebens-mittel- Wissenchaft Und-technologie; 32: 269-277.

Ellman GL (1959). Tissue sulfhydryl groups. Arch Biochem Biophys. 82:70–7.

Gulcin I, Oktay M, Kufrevioglu OI, Aslan A (2002). Determination of antioxidant activity of lichen *Cetraria islandica* (L) Ach. J Ethnopharmacol. 79: 325 - 329.

Gutteridge JMC, Halliwell B (2010). Antioxidants: Molecules, medicines and myths. Biochem Biophy Res Commun. 393, 561-564.

Ghosh A (2003). Herbal folk remedies of Bankura and Medinipur districts, West Bengal (India). Indian J Tradit Knowl. 2: 393-396.

Gomez-Galera S, Pelacho AM, Gene A, Capell T, Christou P (2007). The genetic manipulation of medicinal and aromatic plants. Plant Cell Rep, 26, 1689-1715.

Guyton AC, Hall JE (1998). Textbook of medical physiology. 9th ed. Philadelphia: W.B. Saunders Company, 920-92.

Howard M (1993). Fever: causes and consequences. *Neurosci. Biobehav. Rev.* 17(3):237–269.

Hawaii Powell, Albert Michael (1979). Annals of the Missouri Botanical Garden, 1979; 65(2): 628-629.

Halliwell B (1991). Reactive oxygen species in living systems: Source, Biochemistry and role in human disease. Am J Med, 91, 14-22

Krishnaiah D, Sarbatly R, Nithyanandan R (2010). A review of the antioxidant potential of medicinal plant species. Food Bioprod Process.

Keele CA (1969). Analgesics, section of physical medicine. Proc Roy Sec Med, 62, 535-539.

Kannat SR, Chander R, Sharma A (2007). Antioxidant potential of mint (Mentha spicata L.) in radiation-processed lamb meat. Food Chem, 600, 451-458

Middleton JRE, Kandaswami C, Theoharides TC (2000). The effects of plant flavonoids on mammalian cells: Implication for inflammations, heart disease and cancer. Pharmacol Rev, 52, 673-751.

Mary NK, Shylesh BS, Babu BH, Padikkala J (2002). Antioxidant, and hypolipidaemic activity of a herbal formulation-liposem. Ind J Exp Biol, 40, 901-904..

Maehly AC, Chance B. I (1954). New York: Interscience; Methods of Biochemical Analysis, 357–8.

Moncada A, Palmer RMJ, Higgs EA (1991). Nitric oxide physiology,pathophysiology and pharmacology. Pharmacol Rev, 43,109-142.

Martinez-Vazquez M, Ramirez Apan T.O, Aguilar H, Bye R (1996). Analgesic and antipyretic activities of an aqueous extract and of the flavone linarin of Buddleiacordata. *Planta Med,* 62,137–140.

Ngbede J, Yakubu RA, Nyam DA (2008). Phytochemical screening for active compounds in Canarium schweinfurthii (Atile) leaves from Jos North, Plateau State, Nigeria. Res J Biol Sci, 3(9), 1076- 1078.

Nakayama T (1994). Suppression of hydroperoxide-induced cytotoxicity by polyphenols. Cancer Res, 54, 1991-1993.

Nose K (2000). Role of reactive oxygen species in the regulation of physiological functions. Biol Pharm Bull, 23, 897-903.

Okafor OY, Erukainure OL, Ajiboye JA, Adejobi RO, Owolabi FO, Kosoko SB. Modulatory effect of pineapple peel extract on lipid peroxidation, catalase activity and hepatic biomarker levels in blood plasma of alcohol-induced oxidative stressed rats. Asian Pac J Trop Biomed 2011; 1(1): 12-14.

Perez-Jimenez J, Aranz S, Tabernero M, Diaz-Rubio ME, Serrano J, Goni I (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. Food Res Int, 41, 274-285.

Reanmongkol W, Itharat A, Bouking P (2007). Evaluation of the anti-inflammatory, antinociceptive and antipyretic activities of the extracts from Smilax corbularia Kunth rhizomes in mice and rats (in vivo) *Songklanakarin J. Sci. Technol,* 29, (S1):59–67.

Satoh K (1978). Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta, 90, 37–43.

Suzuki N, Mittler R (2006). Reactive oxygen species and temperature stresses: A delicate balance between signaling and destruction. Physiol Plant, 126, 45-51.

Singh R.K, Acharya S.B, Bhattacharya S. K (2000). Pharmacological activity of Elaeocarpus sphaericus. *Phytother. Res,* 14, 36–39.

Saxena K (2016). Analytical and medicinal properties of ethanolic extract of leaves of carissa carandas. World Journal of Pharmacy and Pharmaceutical Sciences, 5(4), 1683-1690.

Tripati YB, Singh AV, Dubey GP (2001). Antioxidant property of the bulb of *Scilla indica*. Science, 80, 1267-1269.

Toda S, Shirataki Y (1999). Inhibitory effects of *Astragali Radix*, a crude drug in Oriental medicines on lipid peroxidation and protein oxidative modification by copper. J Ethnopharmacol, 68, 331-333.

Vogel HG (2002), editor. Drug discovery and evaluation-Pharmacological assays. 2nd ed. Berlin, New York: Springer Verlag, 759–867.