Evaluation of Antioxidant and Antibacterial activity, Phenol and Proline content of Terminalia catappa

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ABSTRACT

Plants perform vital role for the prosperity of mankind. They have been used for medicinal purposes long before prehistoric period. Terminalia catappa leaves have been known as a folk medicine for the treatment of various diseases. In the present work various parts of T. catappa like Green leaf (GL), Green and Red leaf (GRL), Red leaf (RL), Red epicarp (RE), Green epicarp (GE), Red fruit kernel (RK) and Green fruit kernel (GK) were evaluated for their antibacterial and antioxidant potential. Also Proline and Phenolic content of all these parts was determined. Methanol, acetone, and aqueous extracts of T. catappa leaf, epicarp and kernel were evaluated for antibacterial activity by using agar well diffusion method. Methanolic extracts showed best antibacterial activity. Quantitative determination of Phenol, Proline and Antioxidant activity in leaves, epicarp and kernel of T. catappa was carried out using spectrophotometric method. Catechol, Ascorbic acid and Proline were used as standard for calibration of the Phenols, Antioxidant activity, and Proline content respectively. The leaf (GL, RL) contained maximum amount of Phenols as compared to epicarp and kernel. The antioxidant activity of RK and GK was quite low compared to that of other parts. There was no significant variation in Proline content of leaf, epicarp and kernel. Leaves of T. catappa showed high antioxidant and antibacterial activity, high phenols and proline content as compared to others parts.

Keywords: Antibacterial activity, antioxidant activity, phenol, proline.

INTRODUCTION

In many parts of world especially in rural areas folkloric medicine has been practiced for many centuries due to availability and low cost [1]. Medicinal plants have the potential to synthesize a wide variety of chemical compounds that play a vital role in primary health care system [2]. Currently, application of natural products with therapeutic properties is increasing worldwide. Pathogenic microorganisms are the important agents for many diseases [3]. Potential herbal extracts portray as a bridging agent for novel bioactive molecules. The diversification of medicinal plants makes them a treasure for obtaining novel compounds which can be used in medicinal system as drugs or pilot molecules for invention of new drugs [4].

Terminalia catappa Linn. (Combretaceae) belonging to Southeast Asia [3] is a large deciduous tree [5]. T. catappa (Tropical almond) is a large, spreading tree now distributed throughout the tropics in coastal environments. It can tolerate strong winds and moderately high salinity in the root zone. It grows principally in freely drained, well aerated; sandy soils [6]. The obovate leaves of this plant turn pink-red to red-yellow before falling. Some of the pigments responsible for these changes are violanxanthin, lutein and zeaxanthin. In addition to these pigments leaves also contain violeoxanthin, epoxidelutein and cryptoxabthine. Fruits are produced from about 3 years of age, and the nutritious, tasty seed kernels may be eaten immediately after extraction [6].

Phenolic compounds are aromatic compounds that result from the secondary metabolism of plants [7]. They are one of the most broadly occurring groups of phytochemicals that exhibit antiallergenic, antimicrobial, antiartherogenic, antithrombotic, anti-inflammatory, and vasodilatory and cardioprotective effects [8, 9]. Many phenolic compounds have the calibre to function as antioxidants by scavenging free radicals involved in oxidative processes [10]. Phenolic compounds are always present in the form of glycosides in plant and are rarely present in the free form [11, 12].

It has been reported by earlier researchers that T. catappa leaves were found to possess good antioxidant activity, reducing power and inhibitors of peroxidation [13, 14]. Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, alzheimer's disease andcancer [15]. Antioxidants such as carotenoids, glutathione, as well as antioxidant enzymes like peroxidase, superoxide dismutase and catalase has major role in protection of plant cell under stress conditions [16]. Plants are affected by several kinds of stress which results in accumulation of glycerol, sorbitol, proline, etc. [17, 18]. The leaves, fruit kernel and epicarp of T. catappa undergo colour changes from green to red during the entire life cycle. In this study, changes in antioxidant and antibacterial activity, phenol and proline content was measured during this period of visible change.

MATERIALS AND METHODS

Plant Collection

Both the seeds and leaves of the plant were collected in month of October from the Botanical garden of G. M. Momin Women's College. The plant was authenticated in Blatter Herbarium of St. Xavier's College, Mumbai. The plant specimen matches with the Blatter Herbarium specimen no.16063 of H. Santapau and was identified as Terminalia catappa.

Different parts of the plants used for various investigations are as follows:

GL	:	Green Leaf
GRL	:	Green and Red Leaf
RL	:	Red Leaf
RE	:	Red Epicarp
GE	:	Green Epicarp
RK	:	Red Kernel
GK	:	Green Kernel

Antibacterial Activity

Extract preparation for Antibacterial activity

Aqueous Extraction

Aqueous extract of plant material was made by dissolving 1.5 gm of plant material in 25 mL of D/W by boiling for 2 h and then cooled and filtered with muslin cloth. This filtrate was used for further experiments.

Acetone Extraction

Both seeds and leaves of T. catappa were washed and air dried. They were ground to fine powder using mixer grinder and powdered materials were maintained at room temperature and protected from light. Powdered seeds and leaves (0.25gm) with 12.5 mL of acetone were extracted for 24 h in mechanical shaker at room temperature. Extracts were filtered with filter paper (Whatman No.1) and were stored at 4^{o}C [19]. It was then evaporated at room temperature and redissolved in water (1.5 mL) and analyzed for antimicrobial activity.

Methanol Extraction

The fruits and seeds of T. catappa were collected, washed and air dried. They were ground to fine powder using mixer grinder and powdered materials were maintained at room temperature and protected from light. A fine dried powder of each sample (3g) was extracted using 50 mL of methanol in mechanical shaker at room temperature for 60 min and kept overnight. The extracts were filtered through Whatman No. 1 paper and evaporated to dryness at room temperature. All of the samples were redissolved in distilled water (8 mL) and analyzed for antimicrobial potency.

Organism used

Escherichia coli (E. coli) belonging to family Enterobacteriaceae was used as the test organism.

Agar well diffusion method

The aqueous extract, acetone extract and methanol extracts were used for antibacterial activity. The antibacterial activity was evaluated at a concentration of 50µl/well. Antibacterial activity was performed by agar well diffusion [20]. Luria Bertani Agar was the media used to study the antibacterial susceptibility. The media and test bacterial culture (E. coli) were poured into petri dishes. The sample (50µl) was impregnated in to a well of diameter 0.5 cm followed by incubation of plates at 37°C for 48h. The experiment was performed under aseptic conditions and susceptibility was determined by measuring the zone of inhibition. The experiment was performed in triplicates**.**

Extraction and Detection of Antioxidant Compound

Extract Preparation

The fruits and seeds of T. catappa were collected, washed and air dried. They were ground to fine powder using mixer grinder and powdered materials were maintained at room temperature and protected from light. A fine dried powder of each sample (3 g) was extracted using 50 mL of methanol in mechanical shaker at room temperature for 60 min and kept overnight. The extracts were filtered through Whatman No. 1 paper and evaporated to dryness at room temperature. All of the samples were redissolved in distilled water (8 mL) and analyzed for their contents in terms of antioxidant activity.

Determination of Antioxidant activity

The method described by Prieto et al. in 1999 [21] was adapted to measure the antioxidant capacity of T. catappa extracts. In brief, to a known aliquot of sample solution (0.4 mL) taken in a vial, 4 mL of the reagent solution (0.6 M sulphuric acid, 4M ammonium molybdate) was added and incubated in a water bath at 95^{o}C for 90 min. Absorbance was measured at 695 nm. Calibration curve was prepared by using standard solution of ascorbic acid (5-100 ug mL^{-1}) and the antioxidant activity was expressed as mg AAEAC (Ascorbic acid equivalent antioxidant capacities) per gram extract [22].

Extraction and Detection of Phenols

Extract Preparation

Both seeds and leaves of T. catappa were air dried and used for further experiments. Accurately weighed powder (0.5gm) of sample was ground with mortar and pestle in the measured volume of solvents (10 mL) of 80 ethanol:20 water. Filtered extract was used further.

Determination of Phenols

The total phenol content of each plant extract was estimated by method described by Malik & Singh [23]. Aliquots of extracts (0.01 mL) were taken and final volume (3mL) was made with distilled water. Then 0.5 mL folin-ciocalteau reagent (1:1 with water) and 2 mL Sodium carbonate (20%) were added sequentially in each tube. Blue colour was developed because phenols undergo complex redox reaction with phosphomolibdic acid in folin-ciocalteau reagent in alkaline medium which resulted in blue coloured complex, molybdenum blue. The test solution was warmed for 1 min, cooled and absorbance was measured at 650nm against the reagent used as a blank. Calibration plot was generated using catechol. The concentration of phenols in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol\g of dry material by method given by Khatiwora et al. [24].

Extraction and detection of Proline

Extract Preparation

Both seeds and leaves of T. catappa were extracted (0.5 gm) by homogenizing in 10% of 3% aqueous sulphosalicyclic acid. The filtrate was used for measuring proline content.

Determination of Proline

The total proline content of each plant extract was estimated by method described by Bates et al. [25]. Each sample (2 mL) was taken in a test tube and 2 mL of glacial acetic acid and 2 mL of acid ninhydrin was added. The mixture was then kept on boiling water bath for 1 h. The reaction was then terminated by placing the tube in ice bath. After that 4 mL toluene was added and mixture was thoroughly shaken for 20-30 s. The absorbance of red colour intensity was measured at 520 nm versus the toluene blank. A standard curve was prepared by running a series of standard with pure proline [25].

Statistical Analysis:

All assays were carried out in triplicates and results are presented as Mean $\pm$ SD.

RESULTS AND DISCUSSION

In the present study the parts of T. catappa extracts in methanol, acetone and water were investigated for their antibacterial potential against E. coli strain. The antibacterial activity of methanolic extract was maximum in Green leaf (13 mm) as compared to Red leaf (6 mm) and Green & Red leaf (11.33 mm). No significant difference was found in Green Epicarp (9.5 mm) and Red Epicarp (9.5 mm) (Fig.1a). In acetone extract maximum activity was found in leaf parts i.e. Green & Red leaf (10.33 mm), Red leaf (9.75 mm), and Green leaf (9.6 mm) respectively. In case of Epicarp and kernel there was no activity found in Acetone extract but in Methanolic extract antibacterial activity was found in Epicarp (Fig.1b). In aqueous extract the zone of inhibition was maximum in Red leaf (14 mm) as compared to Green leaf (8.5 mm) and Green & Red leaf (10 mm). Antibacterial activity was maximum in Green Epicarp (10.5 mm) compared to Red Epicarp (9.83 mm). In Kernel there was no activity was found (Fig.1c).

The best antibacterial activity was shown by the methanolic extract in maximum plant parts. The need of the hour is to find new antimicrobials because the microorganisms are getting resistant to the existing antibiotics [26, 27]. Utilization of herbal medicine in treatment of health ailments is gaining popularity in all over the world [28]. Instances of multiple drug resistance in human pathogens have developed due to indiscriminate use of commercial antimicrobial drugs. In addition to this problem, antibiotics are sometimes associated with adverse effect on host including hypersensitivity, immune suppression and allergic reactions [1]. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infections obtained from various sources such as medicinal plant [29, 30].

The results of present study signify the potential of T. catappa leaf as a source of therapeutic agents which provides leads in the ongoing search for antimicrobial botanicals and it also suggest that methanol is the best solvent to extract the active compound. However, the extracts are needed to be checked against some human pathogenic organisms also.

Antioxidant compound is associated with the total phenol content [31]. The plant extracts with higher level of total phenolic and flavonoids also exhibit greater free radical scavenging activity [31, 32]. Antioxidants are the substances that provide protection against oxidative damage [33]. In the present study antioxidant activity was evaluated for T. catappa leaves and parts of seeds (Epicarp and Kernel) (Fig. 2).

The Antioxidant activity of T. catappa was found maximum in Green Epicarp (1.13 mg AAEAC/g extract) followed by Green leaf (1.11 mg AAEAC/g extract), Red Epicarp (1.09 mg AAEAC/g extract), Green & Red leaf (1.07 mg AAEAC/g extract), Red leaf (1.00 mg AAEAC/g extract), Red Kernel (0.65 mg AAEAC/g extract) and Green Kernel (0.42 mg AAEAC/g extract).

Polyphenols present in plants, fruits and vegetables are an important source of natural antioxidants as they act as reducing agents, hydrogen donors, single oxygen quenchers and potential metal chelators [19, 25]. The level of Phenol in different parts of the plant extract is shown in (Fig. 3). The result indicated that the total phenol content of various extract had significant variation. Analysis of the phenolic contents revealed that the Green leaf (GL) contained the maximum phenolic content (23.8 mg catechol equivalent/ g extract) followed by Red leaf (18.5 mg catechol equivalent/ g extract), Green Epicarp (3.23 mg catechol equivalent/ g extract) and Red & green leaf (1.538 mg catechol equivalent/ g extract). There was no Phenolic content found in Red Epicarp (RE), Red Kernel (RK) and Green Kernel (GK). The result indicates that the leaf of T. catappa consist of the maximum phenolic compounds as compared to the other parts of the plants.

Earlier research indicates that T. catappa leaf is rich in polyphenolic compounds. Consumption of fruits, vegetable and plants rich in polyphenols is associated with the reduced risk of certain Cancer, Cardiovascular diseases, Atherosclerosis, Diabetes and Alzheimer's diseases [34, 35, 36]. These properties may be due to its phenolic content. However in the

present study a comparative account is made between different plant parts which suggest that red leaf is also rich in phenolic content. Results show that GL has highest phenolic content and antioxidant activity. Several investigations of antioxidant activity of plant extract have confirmed a high linear correlation between the values of phenol concentration and antioxidant activity.

Proline has also been estimated in the present study. Total proline content in the examined extracts ranged from 0.72 mg proline/ g extract to 0.83 mg proline/ g extract. There was no significant difference in the proline content measured in any of the plant part (Fig. 4). The exogenous proline has been reported to protect plants under stress. Proline, Antioxidant activity and phenol get accumulated under various abiotic stresses (Heat, Cold, Drought, Moisture and Salinity).

Thus here there is a strong correlation found between the presence of secondary metabolites of the plant like proline, antioxidant compound and phenol with the antimicrobial activity tested. The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results so it can be recommended for pharmaceutical importance subjected to further tests.

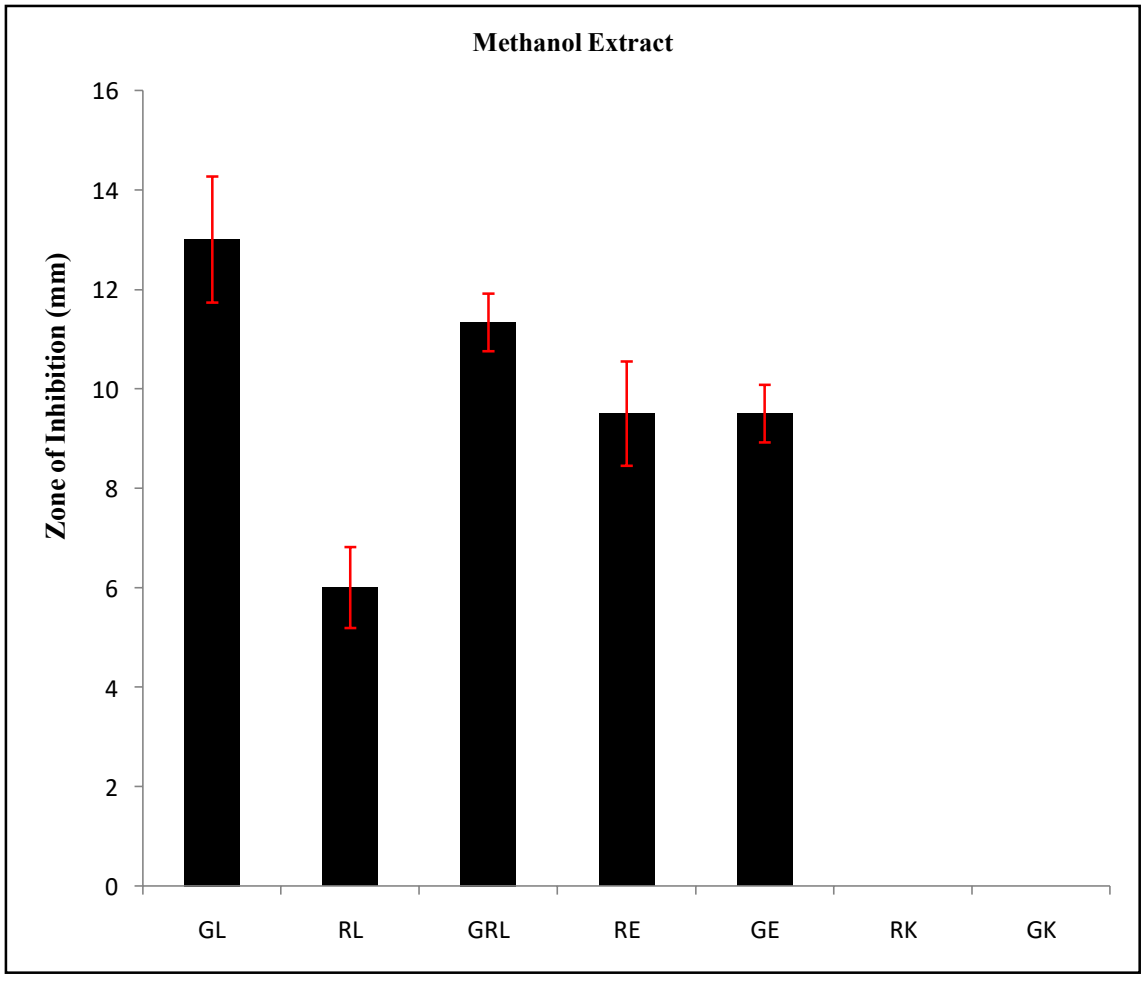

Fig-1a: Antibacterial Activity of Methanol Extract of T. catappa

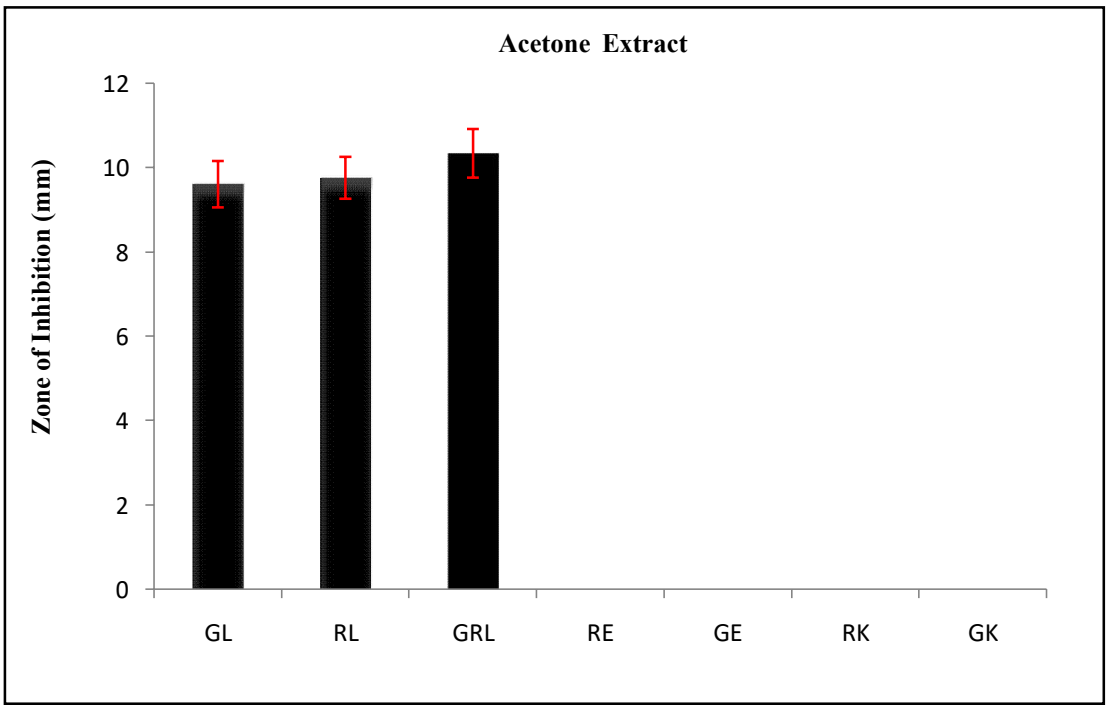

Fig-1b: Antibacterial Activity of Acetone Extract of T. catappa

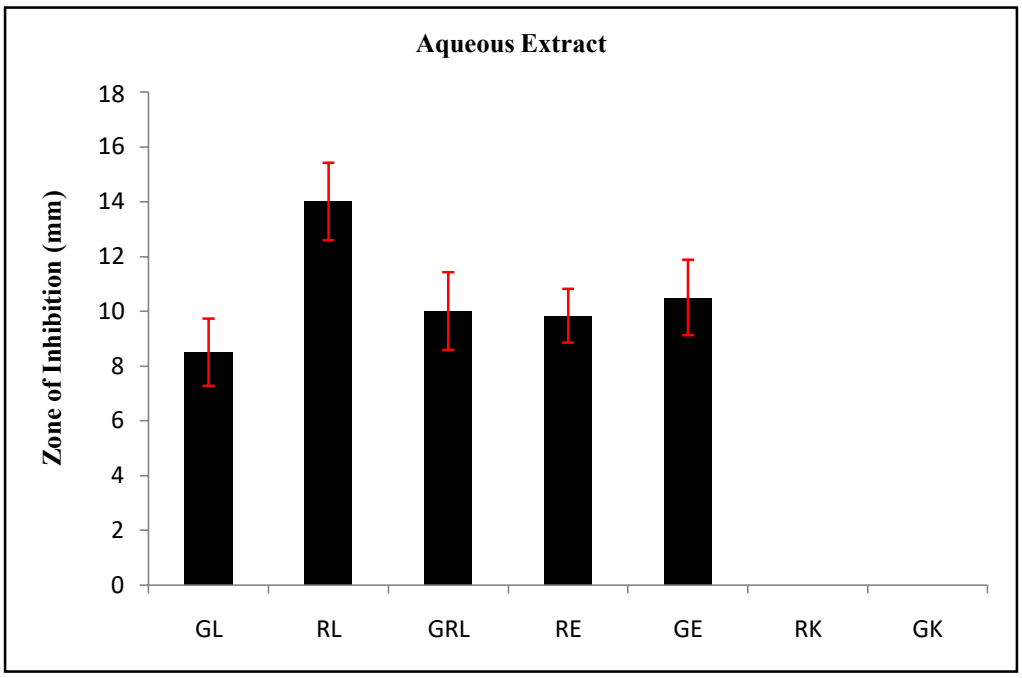

Fig-1c: Antibacterial Activity of Aqueous Extract of T. catappa

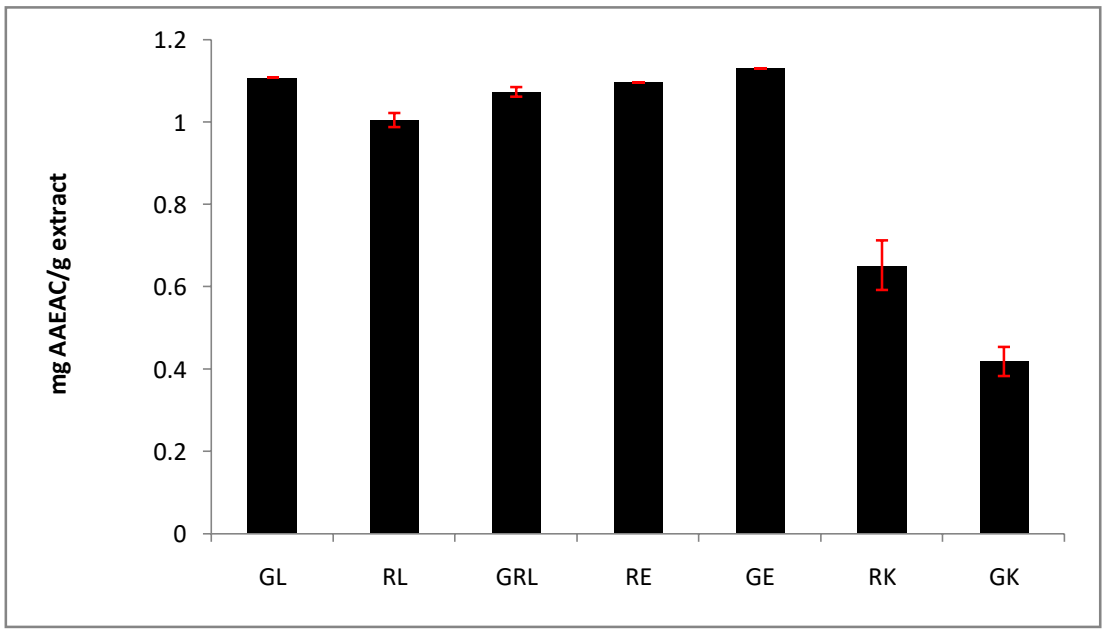

Fig 2: Determination of Antioxidant activity in various parts of T. catappa

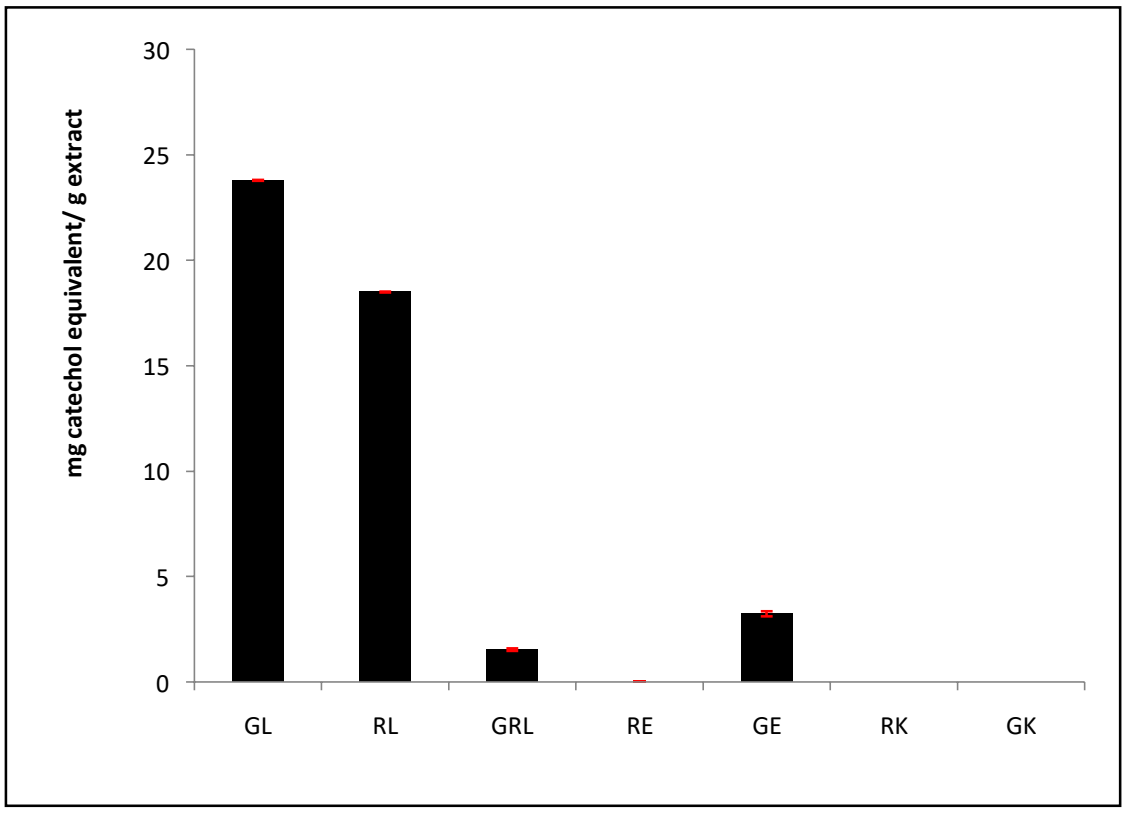

Fig 3: Determination of Phenol contents in various parts of T. catappa

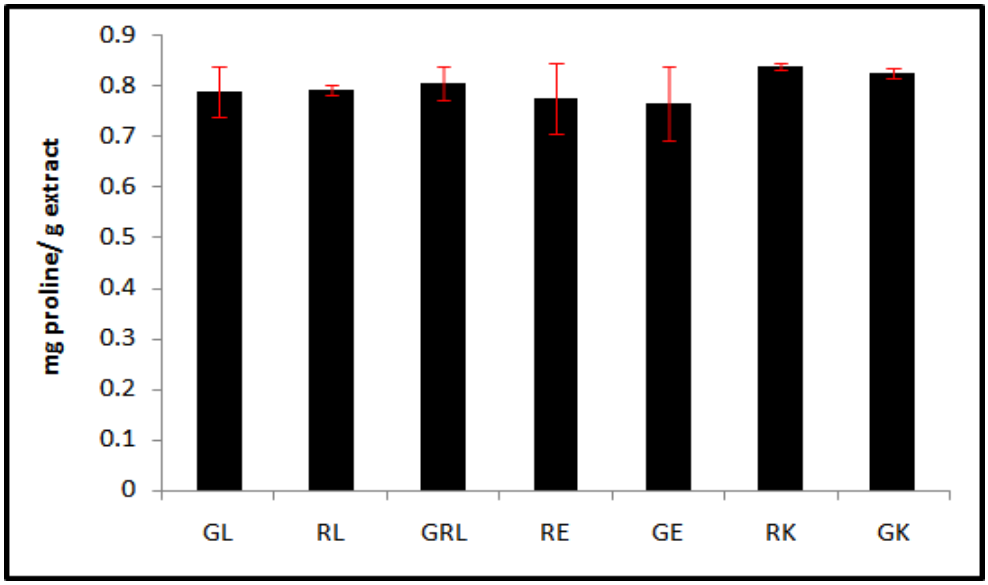

Fig 4: Determination of Proline contents in various parts of T. catappa

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