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COMPARATIVE EVALUATION OF FLAVANOID CONTENT AND ANTITUBERCULAR PERSPECTIVES IN SOME INDIAN MEDICINAL PLANTS

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ABSTRACT

The present research work was aimed to carry out the phytochemical analysis and to investigate the antitubercular perspectives of different extracts of selected Indian medicinal plants such as ***Carica papaya***, ***Psidium guajava***, and ***Punica granatum***. The extraction was carried out by cold maceration technique by using solvents of increasing polarity such as water; ethanol and chloroform and the extractive values were calculated. The flavanoids content was estimated by aluminium chloride method and the results revealed that the ethanol extracts of ***Carica papaya***, ***Psidium guajava*** and ***Punica granatum*** was found to contain 12.50 µg/ml, 11.78 µg/ml and 15.41 µg/ml of flavanoid respectively. The antitubercular activity by Alamar blue assay explored that the ethanol extract of ***Psidium guajava*** exhibit minimum inhibitory concentration of 12.5 µg/ml and the ethanol extracts of ***Carica papaya*** and ***Punica granatum*** was found to have 50 µg/ml and 25 µg/ml as the minimum inhibitory concentration respectively.

INTRODUCTION

India has the heaviest burden of tuberculosis and resistant TB in the world. According to the WHO report of 2019, India had 21.5 lakh cases of tuberculosis. Tuberculosis produces devastating effects on the socioeconomic development mainly in the developing countries due to its association with other diseases like HIV/AIDS and malnutrition. The emerging of the new type of virus like COVID-19 still worsens the control of tuberculosis in developing nations [1].The resistance offered by the organism towards the drugs used in the treatment of tuberculosis made the chemists to explore new molecules to combat the resistance produced by the organism. Literature studies give the evidence that the seed and leaf extract of papaya shown activity against tuberculosis [2]. The leaf extract of guava plant had shown antimicrobial effect [3]. The pomegranate aril extract had shown antimicrobial activity [4]. Nature has given numerous resources to mankind for the betterment of life free from different disease ailments. It plays an important role in maintaining the homeostatis.From the ancient arena, humans were dependent on plants and other natural resources for living day to day life.Food is the medicine quoted by Hippocrates [5]; It is a magical text for leading a healthy life. Folklore medicine aims in curing of various unsung diseases from ancient period.Root decoctions, leaf extracts, volatile oils, seeds, fruits of medicinal plants were used in the treatment of snake poisoning, fever, commom cold and other related diseases.Ancient Egyptians used garlic, onions, radish, honey and lint & lard for the treatment of infections and the dressing of wounds. Indian palm scripts denotes the various traditional system of medicine which has given the access to treat life –threatening chronic diseases.The unsung values of herbal wealth were

still under exploration.In the Modern era of civilization,eventhough many synthetic analogues were been utilised in the treatment of ailments, due to the prevailing drug resistance, medicinal chemist switch over to the exploration of indigenous system of medicine. Hence the demand for rationale drug discovery flourished in order to combat the prevailing vulnerable diseases. Based on the folklore claims, three medicinal plants were selected for study such as ***Carica papaya, Psidium guajava,*** and ***Punica granatum. Carica papaya*** belongs to the family of Caricaceae having much potential used in the treatment of warts, boils and urinary infections. The root decoction is used in expelling of roundworms. Leaf extract of papaya is used in the treatment of the dengue viral infection [6]. ***Psidium guajava,*** a common tropical medicinal plant belongs to the family of Myrtaceae had folklore claims of antimicrobial to anticancer properties [7]. Another fruitful plant ***Punica granatum*** was selected based on their magical health benefits such as prevention of cancer, arthritis and to fight against heart disorders [8]. Based on the literatures, it was proved that there were much beneficial potency lies in the commonly used medicinal plants but some of the biological properties remain untapped. So the research work was aimed to test the potency of the leaf extracts against the dreadful disease such as tuberculosis in a comparable manner and to provide evaluation of the flavanoid content present in the plants.

MATERIALS AND METHODS

Collection of plant specimen: The plant specimen for the proposed study was collected from Venkatramapuram Village, Tirupati. Care was taken to select healthy leaves from the plant which were hand plucked and removed from the plant.

Instrumentation and reagents: Solvents like Analytical grade chloroform, ethanol, methanol and other chemicals were procured from S.D. fine chemicals, Mumbai. Absorbance was taken by using Analytical UV Visible spectrophotometer. Citizen Scale India Pvt ltd- Electronic balance was used. Rotary evaporator manufactured by S.R enterprises, Tirupati was used.

Extraction: The leaves of the three plant ***Carica papaya, Psidium guajava*** and ***Punica granatum*** were washed with water, cut into small into small pieces and shade dried for 2-3 weeks, then they were coarse powdered by passed through sieve no. 40. The powder thus obtained was used for extraction. Cold maceration [9] was rationally selected as the extraction procedure to explore the phytoconstituents present in the medicinal plants. The solvents were selected based on the decreasing order of polarity such as water, ethanol and chloroform. The shade dried coarsely powdered leaves of the three plant (100 g) was extracted by cold maceration technique separately with each solvent for 24-72 hours. After the completion of extraction, it was filtered and solvent was recovered by Rota vap under reduced pressure.The residue was stored in the desiccator.

Qualitative analysis of different extracts: The extracts obtained were subjected to different chemical tests [10] in order to identify the phytoconstituents present in it. The tests were undergone to identify constituents such as alkaloids, tannins, steroids, flavanoids, carbohydrates and terpenoids.

Estimation of total flavanoid content: The estimation of flavanoid was performed as per the protocol described by Chang et al., [11]

Preparation of Standard solution: The estimation of flavanoid is performed by using Quercetin as the standard in the prepared extract. A standard solution 10 µg/ml was made by weighing 1mg of Quercetin and dissolved in 100 ml of methanol.

Preparation of Calibration curve: From the standard stock solution, aliquots of 0.1, 0.2, 0.4, 0.6 0.8 and 1.0 ml was taken in a separate 10ml volumetric flask. To the volumetric flask, 1.5 ml of methanol, 0.1ml of 10% aluminium chloride, 0.1 ml of 1Molar potassium acetate and 2.8 ml of distilled water was added. The mixture was kept aside for half an hour at room temperature and the volume was made up with water. The resulting solution absorbance was measured at 415 nm against reagent blank. The calibration curve was plotted by absorbance versus concentration values and the concentration of flavanoids present in the different leaf extract was determined by using the standard calibration curve.

Antitubercular activity

The antitubercular activity of the extracts was determined by Alamar blue assay method described by Maria et al [12]. The study was conducted at Maratha Mandal's Central Research Centre, Belgaum, Karnataka. This method is non-

toxic utilizes thermally stable reagent and have better protocol correlated with BACTEC radiometric method. Briefly, 200 µl sterile deionzed H_2O was added to sterilize 96 well plates and to reduce the evaporation of the medium during incubation period. Then 100 µl of Middle brook 7H9 broth was added to the plates and the corresponding serial dilutions were made. The testing sample concentrations were made between 100 µg/ml to 0.2 µg/ml. The micro titer plates were sealed with parafilm and kept for incubation at 37ºC for 5 days. After incubation, add 25 µl of freshly prepared Alamar blue reagent and 10% tween 80 in the ratio of 1:1 to the plate and incubated for period of one day. It was further interpreted by observing the color developed in the plate. A blue coloration indicates no bacterial growth and pink color was scored as growth. The MIC was calculated as the lowest drug concentration which prevented the color change from blue to pink. The standard drugs used were streptomycin 6.25 µg/ml, Ciprofloxacin 3.125 µg/ml and Pyrazinamide3.125 µg/ml. The Standard Strain used: ***Mycobacteria tuberculosis*** (Vaccine strain, H37 RV strain): ATCC No- 27294.

RESULTS AND DISCUSSION

The research work on comparative evaluation of flavanoid content and the exploration of phytoconstituents for its antitubercular potency of the three plants ***Carica papaya* L, *Psidium guajava*** and ***Punica granatum*** lead the following results.

Extractive values of the leaf extract: The leaves extract of the medicinal plants ***Carica papaya* L, *Psidium guajava*** and ***Punica granatum*** by using different solvents such as water, ethanol and chloroform was prepared and the extractive values were calculated. The results are tabulated in the table no:1

Qualitative phytochemical analysis of leaf extracts: The Qualitative analysis of plant extract helps in identifying the phytoconstituent present in the medicinal plants. The biological potential of the plant is due to the presence of particular bioactive principle. The various phytoconstituents present in the leaf extracts of ***Carica papaya, Psidium guajava*** and ***Punica granatum*** was explored as follows. The exploration study in the phytoconstituents revealed the presence of alkaloids, glycosides, tannins and flavanoids. The Chemical constituents like alkaloids, flavanoids, and carbohydrates were present in aqueous extracts of ***Carica papaya*.** The Constituents such as alkaloids, tannins, flavanoids and terpenoids were present in ethanol extract of **Carica *papapa*** and alkaloids, glycosides, tannins, steroids were present in chloroform extract of ***Carica papaya*.** The chemical constituents like alkaloids, flavanoids, carbohydrates and glycosides were present in ***Psidium guajava*.** The constituents like alkaloids, flavanoids, tannins and terpenoids were present in ethanol extract of ***Psidium guajava*.** The constituents like alkaloids, glycosides, tannins, steroids and carbohydrates were present in chloroform extract of ***Psidium guajava*.** The chemical constituents like alkaloids, glycosides, flavanoids and carbohydrates were present in aqueous extract of ***Punica granatum*.** The chemical constituents like alkaloids, tannins, flavanoids and terpenoids were present in ethanol extract of ***Punica granatum*.** The chemical constituents like alkaloids, glycosides, tannins, flavanoids and terpenoids were present in chloroform extract of ***Punica granatum*.**, The results were tabulated in the table no :2, 3, 4

Estimation of total flavanoids:

The total flavanoid concentration in the extracts was estimated by using Aluminium chloride method. It was found that the aqueous extract of ***Carica papaya* , *Psidium guajava*** and ***Punica granatum*** was found to contain 18.18 µg/ml, 96.15 µg/ml and 19.33 µg/ml of flavanoid respectively. The ethanol extracts of ***Carica papaya* , *Psidium guajava*** and ***Punica granatum*** was found to contain 12.50 µg/ml, 11.78 µg/ml and 15.41 µg/ml of flavanoid respectively. The estimation of flavanoids revealed that the aqueous extract of ***Psidium guajava*** contain higher concentration of flavanoids compared to the lower concentration of flavanoids present in the other extracts. The results were given in the figure 2.

Antitubercular activity

Based on the literature reviews and by qualitative phytochemical analysis, the ethanol extracts were selected for screening against ***Mycobacterium tuberculosis*** by Alamar blue assay method.

Table no: 1 Extractives value of leaf extracts

S.no	Type of extract	Extractive Value (% W/V)
1	***Carica papaya*** Aqueous	1.2
2	***Carica papaya*** Ethanol	1.5
3	***Carica papaya*** Chloroform	2.0
4	***Psidium guajava*** Aqueous	1.8
5	***Psidium guajava*** Ethanol	1.3
6	***Psidium guajava*** Chloroform	0.82
7	***Punica granatum*** Aqueous	1.6
8	***Punica granatum*** Ethanol	1.7
9	***Punica granatum*** Chloroform	1.9

Table no: 2 Qualitative analysis of leaf extract of *Carica Papaya*

S.no	Chemical Constituent	Aqueous Papaya	Ethanol Papaya	Chloroform Papaya
1	Alkaloids	+	+	+
2	Glycosides	+	-	+
3	Tannins	-	+	+
4	Flavanoids	+	+	-
5	Steroids	-	-	+
6	Carbohydrates	+	-	+
7	Terpenoids	-	+	-

+ = present; - = absent

Table no: 3 Qualitative analysis of leaf extract of *Psidium guajava*

S.no	Chemical Constituent	Aqueous Guava	Ethanol Guava	Chloroform Guava
1	Alkaloids	+	+	+
2	Glycosides	+	-	+
3	Tannins	-	+	+
4	Flavanoids	+	+	-
5	Steroids	+	-	+
6	Carbohydrates	+	-	+
7	Terpenoids	-	+	-

+ = present; - = absent

Table no: 4 Qualitative analysis of leaf extract of *Punica granatum*

S.no	Chemical Constituent	Aqueous Pomegranate	Ethanol Pomegranate	Chloroform Pomegranate
1	Alkaloids	+	+	+
2	Glycosides	+	-	+
3	Tannins	-	+	+
4	Flavanoids	+	+	-
5	Steroids	+	-	+
6	Carbohydrates	+	-	+
7	Terpenoids	-	+	-

+ = present; - = absent

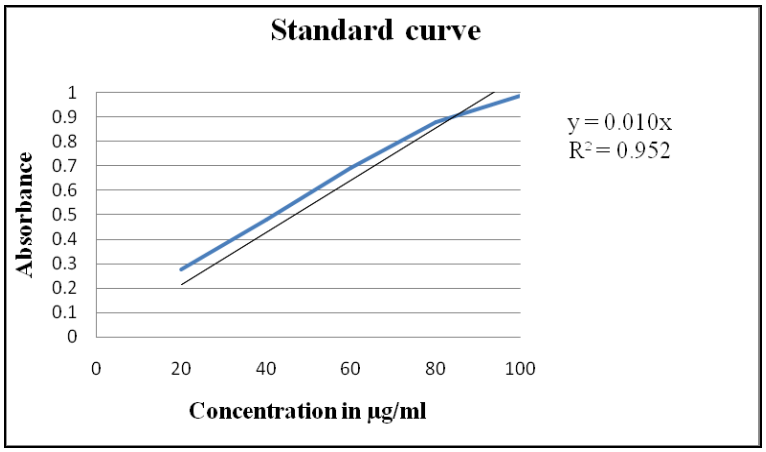

Figure: 1 Calibration curve of total flavanoid estimation

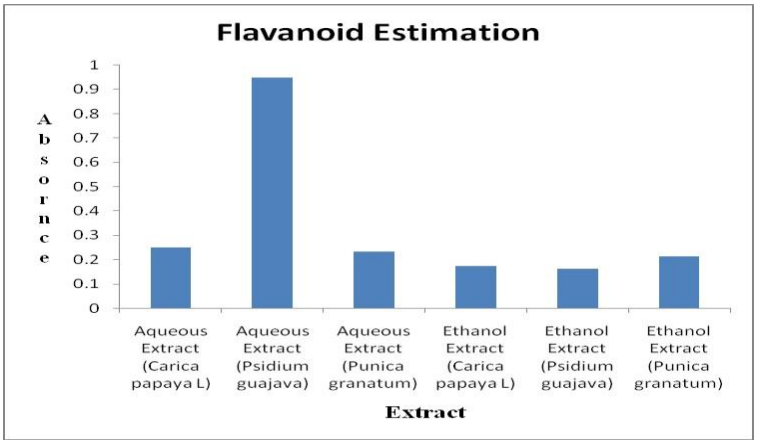

Figure 2: Estimation of total flavanoid in leaf extracts

Table no 5: Anti tubercular screening of ethanol extracts of *Carica papaya* L, *Psidium guajava* and *Punica granatum*

S. No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	TGE	S	S	S	S	R	R	R	R
2	TPAE	S	S	R	R	R	R	R	R
3	TPGE	S	S	S	R	R	R	R	R

TGE: Tubercular guava ethanol; TPAE: tubercular papaya ethanol; TPGE: Tubercular pomegranate ethanol. S-Sensitive R-Resistant

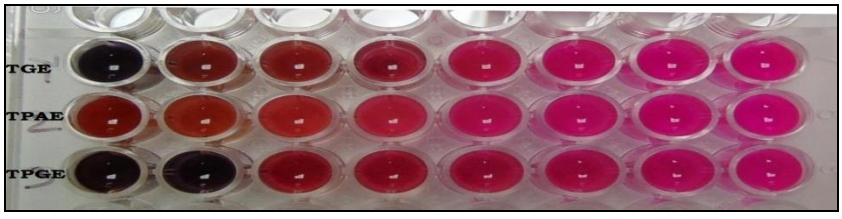

Figure 3: Anti tubercular screening of ethanol extracts of *Carica papaya* L, *Psidium guajava* and *Punica granatum* (TGE: Tubercular guava ethanol; TPAE: tubercular papaya ethanol; TPGE: Tubercular pomegranate ethanol)

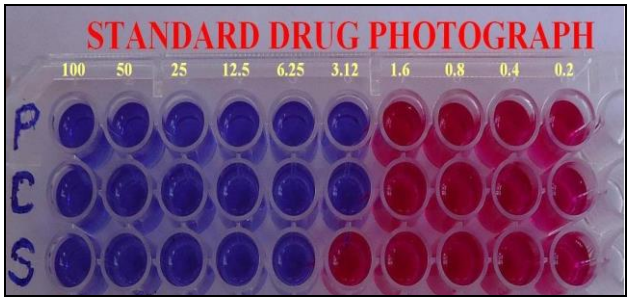

Figure 4: Standard drug used in Antitubercular study.
Standard values for the Anti-Tb test which was performed. P-Pyrazinamide- 3.125µg/ml; C-Ciprofloxacin- .125µg/ml; S-Streptomycin- 6.25µg/ml

The study on antimycobacterial perspectives of ethanol leaf extracts of ***Carica papaya , Psidium guajava*** and ***Punica granatum*** reveals that the ethanol extract of ***Psidium guajava*** has minimum inhibitory concentration of 12.5 µg/ml and the ethanol extracts of ***Carica papaya*** and ***Punica granatum*** was found to be 50 µg/ml and 25 µg/ml respectively. It was also found that leaves of ***Psidium guajava*** has more potency towards antitubercular activity compared to the other plants ***Carica papaya*** and ***Punica granatum.*** The results were given in the table 5 and figure 3.

CONCLUSION

An attempt was made to explore the phytoconstituents present in the different extracts of three important medicinal plants. It was found that the all the plants leaf extract constitutes various phytoconstituents. The study also reveals the estimation of flavanoids for the selected medicinal plants. The need of the flavanoids in pharma, agriculture and food industry still one of the global current research interests. The work explored the presence of flavanoids and provided the comparative data of flavanoids content in various extracts of the medicinal plants ***Carica papaya , Psidium guajava*** and ***Punica granatum*** helps us to rationally select a medicinal plant for the investigation and treatment of illness including degenerative disorders which affects the human population worldwide. The research also revealed the comparative antitubercular potential of the three plants pave the way to explore the much potential plant in showing its biological potency which aids in rationale selection of the active molecules in future.

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