INTRODUCTION

Plants are considered to be the good source for the exploration and discovery of new pharmaceutical compounds as well as medicines, which can be the potential drug for humans as they act as intermediate for synthesis of useful drug [1]. Plants possess various phytochemicals with several bioactivities such as anti-inflammatory, antioxidant, and anticaner [2] and one among them such as Carica papaya is widely used in the treatment of many ailments in Ayurvedic as well as herbal and folk medicine.

C. papaya Linn, is an evergreen shrub or small tree, is a member of family Caricaceae, represented with four genera and four species in India. This plant originated in Southern Mexico and Costa Rica and distributed as a plantation crop in India, Sri Lanka, Hawaii, Australia, and in tropical and subtropical regions [3]. Papaya, the herbaceous perennial plant is also known as Papaya melon tree, Pawpaw or papau, Kapaya, Lapaya, Papyas, Papye, Tapayas, and Fan mu gua. The entire papaya plant possesses various phytomutrients and can be considered for commercial, pharmaceutical, and industrial applications. The papaya plant is best with a large variety of phytomutrients and antioxidant, antimicrobial, and anti-dengue properties [4].

Phytochemicals are the natural, non-nutritive plant chemicals with defensive properties against cancer by protecting the cells from damage. Most of the phytochemicals possess the biological antioxidant capacity that protects our cells against the oxidative damage and reduces the risk of certain types of cancer. These phytochemicals tend to prevent the adhesion of pathogens to the human cell wall by physically binding to it. Some of the important phytochemicals found in C. papaya are Lycopene, Benzylglucosinolate, Beta-carotene, Benzylisothyocynate, chlorogenic acid, caffeic acid, protocatechuic acid, Quercetin, etc. [5,6].

Antioxidants are substances that prevent oxidative damage to the target molecule. An antioxidant can scavenge the free radicals because of their singlet oxygen quenching and redox hydrogen donating features. In recent days, the usage of synthetic antioxidants has been taken over by natural antioxidants as it could be safer without any side effects. In recent decades, due to the various pharmacological actions of the medicinal plants, many researchers are showing interest in studying the antioxidant phytochemicals such as phenols, flavonoids, and tannins which have been recognized for their potential role in preventing human diseases [7-10].

METHODS

Sample collection

Fresh leaves of C. papaya were collected from Mysuru and authenticated by the Department of Water and Health, JSS AHER, Mysore.

Preparation of C. papaya leaf extract

The collected plant materials were washed with running tap water to avoid surface contaminations and shade dried for about 15 days. The dried leaves were cut into small pieces and macerated into a fine powder; dried powder was soaked with different organic solvents such as hexane, ethyl acetate, methanol, and ethanol and was subjected to solvent extraction using the Soxhlet apparatus. Then, the extracted sample was stored at 4°C for further analysis.

Phytochemical analysis

Phytochemical screening was performed for the presence of alkaloids, carbohydrates, amino acids, glycosides, protein, phenolic compounds, and tannins from respective solvents such as hexane, ethyl acetate, methanol, and ethanol, according to standard procedure [11,12].
Stock preparation
200 mg of C. papaya extract was dissolved in 10 ml of each solvent and 1 ml of each solvent was used as a standard for various phytochemical tests.

Alkaloids
Wagner's test – For 1 ml of the sample solution, few drops of Wagner's reagent were added along the sides of the test tube. The appearance of a reddish-brown precipitate indicates the presence of alkaloids [13].

Carbohydrates
Benedict's test – For 1 ml of the sample solution, few drops of Benedict's reagent were added and heated for 2 min. The appearance of a colored precipitate indicates the presence of carbohydrates.

Amino acids
Ninhydrin test – For 1 ml of the sample solution, two drops of ninhydrin reagent were added. The formation of purple color indicates the presence of amino acids.

Glycosides
Kellner-Kiliani test – For 1 ml of sample solution 1 ml of glacial acetic acid, few drops of ferric chloride and concentrated sulfuric acid were added. The appearance of a reddish-brown ring at the junction of liquids indicates the presence of glycosides [13].

Phenolic compounds and tannins
Ferric chloride test – For 1 ml of extract, few drops of neutral 15% ferric chloride were added. The appearance of a dark green color indicates the presence of phenolic and tannin compounds.

Protein
Biuret test – For 1 ml of extract, one drop of 2% copper sulfate and 1 ml of ethanol and potassium hydroxide were added. The presence of the pink color of the ethanolic layer indicates the presence of protein.

Saponins
For 1 ml of extract, few drops of distilled water were added and shaken vigorously. The appearance of foam indicates the presence of saponins [13].

Quinones
For 1 ml of extract, few drops of concentrated hydrochloric acid were added. The formation of a yellow precipitate indicates the presence of quinones.

Oxalate
For 1 ml of extract, few drops of glacial acetic acid were added. The appearance of greenish-black coloration indicates the presence of oxalate.

Anthocyanins
For 1 ml extract, 2 ml of hydrochloric acid and 1 ml of ammonia were added. The color change from pink-red to blue-violet indicates the presence of anthocyanins.

Determination of total phenolic content (TPC)
The TPC of the C. papaya sample was determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method with slight modifications [1 1,1 2].

A standard solution of gallic acid was prepared using the distilled water in the concentration of 1 mg/ml. Different working standards were prepared to obtain the standard calibration curve, followed by diluting with distilled water with 3 ml and 0.5 ml of FC reagent (1:1) and incubated at room temperature for about 15 min, and then 2 ml of 7% sodium carbonate was added. Similar steps were followed for estimating phenolic content in the sample extract and the absorbance was measured at 765 nm against blank using spectrophotometer. All experiments were made in triplicates and the TPC was determined using the standard gallic acid calibration curve.

Determination of total flavonoid content (TFC)
The TPC of the C. papaya sample was determined spectrophotometrically according to the aluminum chloride method with slight modifications [1 1,1 2].

A standard solution of quercetin was prepared using methanol in the concentration of 1 mg/1 ml. Different working standards were prepared to obtain a standard calibration curve using 1 ml with methanol and 0.5 ml of 1.2% AlCl₃, 0.5 ml of sodium acetate and incubated for 30 min. Similar steps were followed for estimating flavonoid content in the sample extract and absorbance was measured at 415 nm against blank using spectrophotometer. All determinations were made in triplicates and the TFC was determined using the standard quercetin calibration curve.

Antioxidant assay
2,2,1-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity
DPPH
The free-radical scavenging activity was determined by DPPH proposed by Zadeh et al., 2008, with slight modifications [1 4].

A standard solution of ascorbic acid was prepared in the concentration of 2 mg/ml in methanol. The standard calibration curve was obtained using different working standards and was made up of 3 ml with methanol. DPPH solution (500 µl) was then added and mixed vigorously. Similar steps were followed for the sample extract. The reaction mixture was incubated for about 45 min in dark condition and absorbance was measured at 517 nm using a spectrophotometer. All determinations were made in triplicates and the standard curve was obtained using ascorbic acid.

The % DPPH which was scavenged (% DPPHₐ) was calculated using the formula:

\[
\text{Scavenging effect (\%)} = \frac{\text{Absorbance of sample at 517 nm}}{\text{Absorbance of control at 517 nm}} \times 100
\]

RESULTS
Qualitative analysis conducted to evaluate the phytochemical profile of C. papaya leaves extract.

Phytochemical screening of C. papaya leaves extracts shows (Figs. 1-4), the presence of alkaloids, proteins, glycosides, phenol, tannin, saponin, quinine, oxalate, and anthocyanin. The presence of alkaloid, carbohydrate, glycoside, phenols, tannin, saponin, and oxalate shows the greater intensity of their presence in methanolic, ethanolic, hexane, and ethyl acetate extract (Table 1). In the methanolic extract, all the bioactive compounds such as alkaloids, glycosides, phenols, tannin, saponin, quinine, and oxalate are present in equal amounts.

The overall result shows that methanol extracts possess a greater number of bioactive compounds when compared to other solvents.

TPC
The total phenol content was determined by Folin-Ciocalteu method and reported as gallic acid equivalents (GAE) concerning the standard curve. The standard taken was gallic acid in a concentration of 1 mg/ml. The concentration of sample extract was evaluated by comparing it to the standard curve. The total phenol content was determined by Folin-Ciocalteu method and reported as gallic acid equivalents (GAE) concerning the standard curve.
The TFC was determined by aluminum chloride method and represented as quercetin equivalents (QAE) concerning the standard curve. The standard taken was quercetin in a concentration of 1 mg/ml. The concentration of sample extract was evaluated by comparing it to the standard Graph 2.

The concentration of flavonoids presents in the leaf extract of *C. papaya* in methanol extract is 21.60 mgQAE/g, hexane extract is 26.48 mgQAE/g, ethyl acetate extract is 27 mgQAE/g, and ethanol extract is 15.65 mgQAE/g extract, respectively, to the QAE of the sample [Table 2].

**Antioxidant activity of *C. papaya***

**DPPH assay** DPPH radical scavenging activity

IC<sub>50</sub> (Inhibitory Concentration) of the sample extract was determined by comparing it to the standard value of ascorbic acid. The result of the antioxidant activity of *C. papaya* with methanolic extract by DPPH assay shows that the presence of free radicals is more and directly proportional to the concentration of the sample. It means higher the concentration higher will be the percentage of free radicals in the *C. papaya* leaves methanolic extract.

The Graph 3 indicates that the percentage of radical scavenging activity of the methanolic extracts at different concentrations with 50% of DPPH scavenging activity was found to be 90 µg/ml, as the concentration of the sample extract was evaluated compared to the standard value. The results show that methanolic solvent extracts analyzed for the DPPH scavenging activity show the higher radical inhibition activity which can be comparable to standard ascorbic acid.

**DISCUSSION**

Medicinal plants constitutes as an important natural wealth of the country by playing a significant role in primary health of mankind. They importantly serve as raw material for manufacturing medicines as therapeutic drugs [15]. *C. papaya* is used as a natural medicinal plant, recognized for its antimicrobial, anti-amoebic, antifungal, and hypolipidemic activity. Mainly papaya is recognized as “King of medicine,” due to the presence of a large number of important phytonutrients loaded in it, and also referred to as “Powerhouse of nutrients”[16,17].

The plant based medicines which have been used in treatment from ancient times reveals in some cases desirable effect were not achieved because the biological action of the herbal medicine of phytoconstituents may vary, as well as the amount of phytoconstituent in a plant can vary according to the age of plant, time of collection, and also environmental conditions [18].

An attempt to study the *in vitro* antioxidant, antimicrobial, and anti-inflammatory of *C. papaya* seed extract with methanolic fractions,
revealed the TPC and the TFC in the methanolic fraction was high compared to other solvent extracts, the antioxidant activity showed the radical scavenging activity of methanolic extracts at different concentration with 50% of scavenging activity, a comparison of the ability of various seed extract have proved their limited DPPH scavenging activity compared to methanol [19].

The antioxidant activity was conducted to compare the total antioxidant activity, TPC, and TFC from the different parts of the papaya tree including their ripe and unripe fruit, seeds, and the young leaves. The result showed that the highest antioxidant activity through  β-carotene assay was observed in unripe fruit (90.67±0.29%), followed by young leaves, ripe fruit, and the seed. In brief, taken to account all the parameter measured, antioxidant was highly remarkable in the sequence of young leaves>unripe fruit>seed [20].

The investigation reports of quantitative analysis of total phenolic and TFCs in extracts of Oroxylum indicum L. Kurz. reveal that the TPC and TFC varied among the different solvent extracts used and among them methanolic extracts of seed showed the highest amount compared to other solvent extracts and insists their ratio and distribution in different parts of the tree that could be used for treating various ailments [2].

An increase in the demand for the natural bioactive compounds from the medicinal plants has provided an opportunity for the extraction of either pure or standardized extracts for various therapeutic activities. C. papaya leaves are a boon from nature for food as well as therapeutic applications.

CONCLUSION

Bioactive compounds can be studied by extraction and isolation, also with defining their structure and by analyzing it in laboratory models as in vitro and in vivo studies and importance was given for identification and characterization of the specific phytochemical which is primarily responsible for biological activity.

The current study which was aimed at investigating the presence of biologically active phytochemicals and antioxidant properties of C. papaya leaves extract reveals that samples with various solvents such as methanol, ethanol, hexane, and ethyl acetate have shown the presence of phytochemicals constituents such as alkaloid, carbohydrates, and amino acids.

Among the used solvent extracts, the C. papaya leaves with methanolic fraction showed the presence of more phytochemicals and have effective phenolic, flavonoid content, and exhibited strongest antioxidant properties which can effectively scavenge reactive oxygen species compared to other solvent extracts used.

Hence, C. papaya can be considered as an important and potential natural source for various pharmaceutical and medicinal applications. Interestingly, the broad spectrum of phytochemicals and antioxidants presents in them can be regarded as the reservoir of naturally occurring diverse bioactive molecules and papaya as herbal medicine can be furnished for the quantitative and qualitative extraction for exploring the new promising biomolecules for pharmaceutical applications.
AUTHOR CONTRIBUTION
Nandini G and Kanthesh BM conceptualized the study. Nandini G and Nagalambika Prasad conducted the experiments. Nandini G drafted the Manuscript. Gopenath G and Murugesan Karthikeyan, Ashok Gnanasekar, Ranjith MS, and Pradeep Palanisamy helped with the Manuscript and Discussion.

DECLARATION AND COMPETING INTEREST
The authors declare that there are no conflicts of interest.

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