INTRODUCTION
The matter of proper identification and appropriate quality that is lack of adulteration, sophistication, or substitution is an extremely important one in the field of herbal medicine. Nowadays, widely used herbs were once the subject of official monographs of the United States Pharmacopeia and the National Formulary. These monographs established legal standard of identity and subject to the limitations of the methods, period, and quality of the herbal drugs.

The basis of standardized extract gives a solid platform for scientific validation of herbs. Plant materials isolated from them show a substantial proportion of world drug market and globally recognized the guidelines for the assessment of quality are necessary. The quality of medicinal plant for therapeutic purpose must be very high. However, it is very difficult to assay for specific chemical entity provided that the bioactive ingredient is unknown [1,2].

Plants are the potential source of natural antioxidants. Natural antioxidants or phytochemical antioxidants are the secondary metabolites of plants [3]. Plant-derived antioxidants have received greater attention since they act as radical scavengers. To the best of our knowledge, no in vitro antioxidant assay of Anacardium occidentale, Achyranthes aspera, and Aegle marmelos of dried bark, whole aerial parts, and leaves revealed the presence of flavonoids, phenols, tannins, and proteins. It also contains alkaloids and glycosides. Hydroalcoholic extract of Anacardium occidentale, A. aspera, and A. marmelos showed highest TPC 0.125 mg/g, 0.256 mg/g, and 0.254 mg/g, respectively. TFCs 0.094 mg/g, 0.145 mg/g, and 0.121 mg/g and highest DPPH RSA with the half maximal inhibitory concentration of 125 μg/mL, 105.58 μg/mL, and 98.09 μg/mL compare to the standard ascorbic acid of 21.65 μg/mL.

RESULTS
The phytochemical screening of A. occidentale, A. aspera, and A. marmelos of dried bark, whole aerial parts, and leaves revealed the presence of flavonoids, phenols, tannins, and proteins. It also contains alkaloids and glycosides. Hydroalcoholic extract of Anacardium occidentale, A. aspera, and A. marmelos showed highest TPC 0.125 mg/g, 0.256 mg/g, and 0.254 mg/g, respectively. TFCs 0.094 mg/g, 0.145 mg/g, and 0.121 mg/g and highest DPPH RSA with the half maximal inhibitory concentration of 125 μg/mL, 105.58 μg/mL, and 98.09 μg/mL compare to the standard ascorbic acid of 21.65 μg/mL.

CONCLUSION
This study showed that the hydroalcoholic extract of A. occidentale, A. aspera, and A. marmelos is potential source of natural antioxidants.

Keywords: Antioxidant activity, Flavonoids, Phenols, Anacardium occidentale, Achyranthes aspera, Aegle marmelos.

MATERIALS AND METHODS
Chemicals and reagents
All the chemicals used were of analytical grade. The chemicals were purchased from Sigma-Aldrich, Bangalore, Global Chemie (India), and Qualikems (India). Sterile distilled water was used, whenever required for the experiment.

ABSTRACT
Objective: Anacardium occidentale, Achyranthes aspera, and Aegle marmelos are the common plants found in North and South India. The present study identifies the active phytochemicals and antioxidant properties in various extracts derived from dried bark, whole aerial parts, and leaves of the above plants.

Materials and Methods: Phytochemical screening included extracts of pet ether, hydroalcoholic, and aqueous. The antioxidant activity was determined by measuring total phenolic contents (TPC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (RSA), and total flavonoid content (TFC).

RESULTS
The phytochemical screening of A. occidentale, A. aspera, and A. marmelos of dried bark, whole aerial parts, and leaves revealed the presence of flavonoids, phenols, tannins, and proteins. It also contains alkaloids and glycosides. Hydroalcoholic extract of Anacardium occidentale, A. aspera, and A. marmelos showed highest TPC 0.125 mg/g, 0.256 mg/g, and 0.254 mg/g, respectively. TFCs 0.094 mg/g, 0.145 mg/g, and 0.121 mg/g and highest DPPH RSA with the half maximal inhibitory concentration of 125 μg/mL, 105.58 μg/mL, and 98.09 μg/mL compare to the standard ascorbic acid of 21.65 μg/mL.

CONCLUSION
This study showed that the hydroalcoholic extract of A. occidentale, A. aspera, and A. marmelos is potential source of natural antioxidants.
extracts and standard solutions were measured against the reagent blank at 750 nm with an ultraviolet (UV)/visible spectrophotometer (UV-1800, Shimadzu, Japan). The TPC was determined from the calibration curve and expressed as milligram of gallic acid equivalent (GAE) per gram of the extracts [6]. The determination of the total phenolic in the extract was carried out in triplicate.

**Determination of total flavonoid content (TFC)**

Aluminum-chloride colorimetric assay was used to determine the TFC in the extracts as previously reported [7]. About 1 mL of the extract (1 mg/mL) was mixed with 4 mL of distilled water in a 10 mL volumetric flask. About 0.3 mL of 5% sodium nitrite was added to the flask. After 5 min, 0.3 mL of 10% AlCl₃ solution was added to the mixture, followed by addition of 2 mL of 1 M NaOH after another 5 min and diluted to the mark with distilled water. A set of standard solutions of quercetin was prepared in the same manner as described for the extracts. The absorbances of the extracts and standard solutions were measured against the reagent blank at 510 nm with a UV/visible spectrophotometer. The TFC was determined from the calibration curve and expressed as milligram of quercetin equivalent (QE) per gram of extracts [8]. The determinations of total flavonoid in the extracts and standards were carried out in triplicates.

**Qualitative phytochemical analysis of the extracts**

Phytochemical analysis of the plant extracts was done to determine the presence of flavonoids, phenol, protein, carbohydrate, alkaloid, and glycoside according to standard methods [9].

**Determination of antioxidant capacity**

The antioxidant capacities of the plants of different extract were studied by the evaluation of the free radical scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) by the modified method [10]. Various concentrations (20–100 µg/mL) of plant extracts (0.3 mL) were mixed with 2.7 mL of appropriate solvent (methanol) containing DPPH radical (0.1 mM). The radical stock solution was prepared fresh daily. Ascorbic acid was used as standard in 20–100 µg/mL solution. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 60 min. The decrease in absorbance of the resulting solutions was monitored at 517 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the equation: % RSA = (As/As/Ao + 1) * 100 [11].

Where, As = Absorbance of the solution when the sample extracts were added at a particular level and Ao = Absorbance of DPPH radical solution. All determinations were performed in triplicate. The half maximal inhibitory concentration (IC₅₀) was calculated as the amount of antioxidants present in the sample necessary to reduce the initial DPPH concentration by 50%.

**Statistical analysis**

All experimental measurements were carried out in triplicate and are expressed as average of three analyses ± standard deviation. The direction and magnitude of correlation between variables was done using analysis of variance (ANOVA) and quantified by the correlation factor.

**RESULTS**

A small portion of the dried extracts was subjected to the phytochemical test identification of carbohydrates, alkaloids, steroids, glycosides, tannins, saponins, flavonoids, proteins, and starch separately for extracts of all samples. Small amount of each extract was suitably centrifuged into the sterile distilled water to make the concentration of 1 mg/ml. The outcomes of the results are discussed in Tables 1-3.

From the results obtained, it is clear that A. occidentale, A. marmelos, and A. aspera hydroalcoholic extract shows the presence of protein, phenol, amino acid, steroid, and flavonoids. Flavonoids and phenol are the phytochemicals that are present in all the hydroalcoholic extracts. Hydroalcoholic extracts of A. occidentale, A. marmelos, and A. aspera were found highly rich in flavonoids and phenols.

**TPC estimation**

Phenolic compounds are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipooxygenase, and scavenge free radicals. The amount of total phenol was determined with the Folin–Ciocalteu reagent.

The content of TPC was expressed as mg/g of GAE of dry extract sample using the equation obtained from the calibration curve: Y = 0.038X + 0.001, R² = 0.997, where X is the absorbance and Y is the GAE.

Gallic acid was used as a standard compound and the total phenols were expressed as mg/g GAE using the standard curve equation: Y = 0.038X + 0.001, R² = 0.997, where X is absorbance at 750 nm and Y is the content in mg/g GAE.

**Table 1: Phytochemical constituents of Aegle marmelos.** "+" indicates, presence; and "−" indicates, absent

| Extracts  | Alkaloids | Glycosides | Phenols/tannins | Flavonoids | Saponins | Carbohydrates | Fats and oil | Steroids | Proteins |
|----------|-----------|------------|-----------------|------------|----------|--------------|-------------|----------|----------|
| Hydroalcoholic | +         | +          | +               | +          | +        | −            | −           | +        | +        |
| Aqueous   | −         | +          | +               | +          | −        | +            | −           | −        | +        |
| Pet ether | −         | −          | −               | −          | −        | −            | +           | −        | −        |

**Table 2: Phytochemical constituents of Achyranthes aspera.** "+" indicates, presence; and "−" indicates, absent

| Extracts  | Alkaloids | Glycosides | Phenols/tannins | Flavonoids | Saponins | Carbohydrates | Fats and oil | Steroids | Proteins |
|----------|-----------|------------|-----------------|------------|----------|--------------|-------------|----------|----------|
| Hydroalcoholic | +         | +          | +               | +          | +        | −            | −           | +        | +        |
| Aqueous   | −         | +          | +               | +          | −        | −            | −           | −        | −        |
| Pet ether | −         | −          | −               | −          | −        | −            | +           | −        | −        |

**Table 3: Phytochemical constituents of Anacardium occidentale.** "+" indicates, presence; and "−" indicates, absent

| Extracts  | Alkaloids | Glycosides | Phenols/tannins | Flavonoids | Saponins | Carbohydrates | Fats and oil | Steroids | Proteins |
|----------|-----------|------------|-----------------|------------|----------|--------------|-------------|----------|----------|
| Hydroalcoholic | +         | +          | +               | +          | −        | −            | +           | +        | +        |
| Aqueous   | −         | −          | −               | −          | −        | +            | −           | −        | −        |
| Pet ether | −         | −          | −               | −          | −        | −            | +           | −        | −        |
Y is TPC in the extracts of A. occidentale, A. marmelos, and A. aspera expressed in mg/g. Table 4 shows the variation of mean absorbance with concentration of gallic acid and shows the calibration curve of standard gallic acid. The contents of total phenols that were measured by Folin-Ciocalteu reagent in the contents of total flavonoids were measured by AlCl₃ reagent in terms of QE.

**TFC estimation**

TFC was calculated as QE (mg/g) using the equation based on the calibration curve: Y=0.041X+0.022, R²=0.995, where X is the absorbance and Y is the QE. Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phytoconstituents.

The amount of total flavonoids was determined with the quercetin. Quercetin was used as a standard compound and the total flavonoids were expressed as mg/g QE using the equation: Y=0.041X+0.022, R²=0.995, where Y is absorbance at 510 nm and X is TFC in the extracts of A. occidentale, A. marmelos, and A. aspera expressed in mg/g. Table 5 shows the variation of mean absorbance value with different concentration of quercetin reagent and shows the calibration curve of quercetin. The contents of total flavonoids were measured by AlCl₃ reagent in terms of QE.

The results obtained from the present study showed that the hydroalcoholic extracts of A. occidentale, A. marmelos, and A. aspera which contains highest amount of flavonoids and phenolic compounds, thus can be used to explore new drugs.

**Antioxidant activity of hydroalcoholic extract**

DPPH scavenging activity is being used by various researchers. It is very easy and reliable parameter for screening the in vitro antioxidant activity of plant extracts. DPPH is a stable free radical and accepts an electron to become a stable diamagnetic molecule. The absorption maximum of a stable DPPH radical in methanol was at 517 nm in UV spectrophotometer. It was observed that with the increase of concentration, there is decrease of absorbance value. The decrease in absorbance of DPPH radical caused by antioxidants, due to the reaction between antioxidants molecules and radical, progresses, which results in the scavenging of the radical by electron donation.

**Table 4: Preparation of gallic acid calibration curve**

| S. No. | Concentration (μg/ml) | Absorbance (Mean) λ max=750 nm |
|--------|-----------------------|-------------------------------|
| 1      | 1                     | 0.041                         |
| 2      | 2                     | 0.073                         |
| 3      | 3                     | 0.115                         |
| 4      | 4                     | 0.154                         |
| 5      | 5                     | 0.210                         |
| 6      | 6                     | 0.231                         |
| 7      | 7                     | 0.273                         |
| 8      | 8                     | 0.301                         |
| 9      | 9                     | 0.342                         |
| 10     | 10                    | 0.389                         |

**Table 5: Preparation of quercetin calibration curve**

| S. No. | Concentration (μg/ml) | Absorbance (Mean) λ max=510 nm |
|--------|-----------------------|-------------------------------|
| 1      | 1                     | 0.071                         |
| 2      | 2                     | 0.115                         |
| 3      | 3                     | 0.155                         |
| 4      | 4                     | 0.195                         |
| 5      | 5                     | 0.235                         |
| 6      | 6                     | 0.275                         |
| 7      | 7                     | 0.312                         |
| 8      | 8                     | 0.351                         |
| 9      | 9                     | 0.391                         |
| 10     | 10                    | 0.438                         |

The percentage of different concentration of standard ascorbic acid and A. occidentale, A. marmelos, and A. aspera is shown in Table 9.

**DISCUSSION**

Phytochemical screening is done for analyzing secondary metabolites, which are responsible for curing ailments. Phytochemical screening of the extracts was investigated according to the standard procedure. The petroleum ether, hydroalcoholic, and aqueous extract of bark of A. occidentale and leaves of A. marmelos and whole aerial plant material of A. aspera were investigated to preliminary phytochemical screening for the presence of various phytoconstituents, i.e. alkaloids, terpenoids, steroids, flavonoids, carbohydrates, proteins, amino acids, tannins, and phenolic compounds present in them.

The results obtained it is clear that all selected plant extracts show the presence of alkaloids, phenols, and flavonoids, in petroleum ether extract shows the presence of only fats and oil. Hydroalcoholic extract of A. occidentale, A. marmelos, and A. aspera shows the presence of alkaloids, glycoside, phenols, and flavonoids.

Quantitative analysis is an important tool for the determination of the quantity of phytoconstituents present in plant extracts. For this, TPC and TFC are determined. The hydroalcoholic extract obtained from bark of A. occidentale, leaves of A. marmelos, and whole aerial plant material of A. aspera were investigated to preliminary phytochemical screening for the presence of various phytoconstituents, i.e. alkaloids, terpenoids, steroids, flavonoids, carbohydrates, proteins, amino acids, tannins, and phenolic compounds present in them.

**Table 6: Total phenolic and flavonoids content of Anacardium occidentale**

| S. No. | Plant extracts | Total phenol (mg/g) | Total flavonoids (mg/g) |
|--------|----------------|---------------------|-------------------------|
| 1      | Hydroalcoholic | 0.125               | 0.094                   |
| 2      | Aqueous        | 0.048               | 0.075                   |

**Table 7: Total phenolic and flavonoids content of Aegle marmelos**

| S. No. | Plant extracts | Total phenol (mg/g) | Total flavonoids (mg/g) |
|--------|----------------|---------------------|-------------------------|
| 1      | Hydroalcoholic | 0.254               | 0.121                   |
| 2      | Aqueous        | 0.121               | 0.095                   |

**Table 8: Total phenolic and flavonoids content of Achyranthes aspera**

| S. No. | Plant extracts | Total phenol (mg/g) | Total flavonoids (mg/g) |
|--------|----------------|---------------------|-------------------------|
| 1      | Hydroalcoholic | 0.256               | 0.145                   |
| 2      | Aqueous        | 0.110               | 0.075                   |
material of A. aspera is subjected to estimate the presence of TPC and TFC by standard procedure.

Phenolics play an important role in plant development, particularly in lignin and pigment biosynthesis. They provide structural integrity and scaffolding support to plants. In this study, the higher amount of TPC was recorded in the hydroalcoholic extract of A. occidentale, A. marmelos, and A. aspera (0.125 mg/g, 0.254 mg/g, and 0.256 mg/g) followed by aqueous extract (0.048 mg/g, 0.121 mg/g, and 0.110 mg/g), respectively [13].

Flavonoids play an important role in the protection of plants against plant-feeding insects and herbivores. Their presence can alter the palatability of the plants and reduce their nutritive value, decrease digestibility, or even act as toxins in few cases. The present work shows maximum TFC in the hydroalcoholic extract of A. occidentale, A. marmelos, and A. aspera (0.094 mg/g, 0.121 mg/g, and 0.145 mg/g) followed by aqueous extract (0.075 mg/g, 0.095 mg/g, and 0.082 mg/g), respectively.

Antioxidant plays a central role in defending free radical species which are formed from various biochemical reactions in normal system. These free radicals are the main culprits in lipid peroxidation. The production of reactive oxygen species and free radicals occurs in various diseases, which directly/indirectly activates phagocytic cells. The synthetic medicines are known to produce severe side effects in the body. Phytodrug having antioxidant property is non-toxic or may have minimum side effects than synthetic compounds. The hydroalcoholic extract of A. occidentale, A. marmelos, and Achyranthes shows the IC50 value of 125 µg/mL, 98.89 µg/ml, and 105.58 µg/mL respectively, and IC50 value for standard ascorbic acid was found to be 21.65 µg/mL. The antioxidant activity of sample was less than that of standard ascorbic acid [14].

CONCLUSION

From our studies, the extracts of medicinal plants were investigated for their total antioxidant capacity; the presence of high phenolics and flavonoids content. The high contents of phenolic compounds indicated that these compounds contribute to the strong antioxidant activity. Further studies of this plant species should be directed to carry out in vivo studies of its medicinal active components to prepare natural pharmaceutical products of high value.

AUTHORS’ CONTRIBUTIONS

Sushil Kumar supervised the work; Brijyog and L. P. Singh conducted the experiments and monitored the work progress. Brijyog and Shweta Verma drafted the manuscript for publication. All authors read and approved the final manuscript.

Table 9: Result of in vitro free RSA

| Concentration (µg/mL) | Ascorbic acid | Hydroalcoholic extracts |
|-----------------------|---------------|-------------------------|
|                       | % inhibition  | Acanthium occidentale   | Aegle marmelos | Achyranthes aspera |
| 20                    | 92.197        | 58.996                  | 78.184        | 87.579          |
| 40                    | 92.914        | 63.535                  | 79.777        | 90.047          |
| 60                    | 93.789        | 66.321                  | 82.882        | 91.082          |
| 80                    | 96.417        | 71.815                  | 84.235        | 92.436          |
| 100                   | 97.452        | 75.00                   | 87.579        | 94.028          |

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest, financial, or otherwise.

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