Research Article

Preliminary phytochemical screening, quantitative estimation and *in-vitro* antioxidant activity of *Anamirta cocculus* (L) fruits

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Abstract

Objective: Plants are important sources of biological activities natural yields, the present study was considered to evaluate the physicochemical, preliminary phytochemical, quantitative and antioxidant activity of hydroalcohol extract of *Anamirta cocculus* fruits (HEAC). **Material and methods:** The Physicochemical properties were carried out from crude powder, such as total ash, acid insoluble ash, water-soluble ash, extractive values and loss on drying (LOD). The HEAC fruits was subjected to phytochemical investigation. Quantitative estimation of total phenolic and flavonoids content was estimated by Folin ciocalteu and aluminium chloride by colorimetric method. *In vitro* antioxidant were analysed by using DPPH assay, hydroxyl radical scavenging, and lipid peroxidation activity. **Results:** Phytochemical analysis exposes the accessibility of ancillary various metabolites are alkaloids, phytosteroids, glycosides, flavonoids, tannins, saponins and phenolic compounds in the extract. The quantitative analysis for HEAC fruits was done for total phenolic content (TPC), total flavonoids content (TFC) were found to be 16.16 ± 0.085 mg of Gallic acid(GA) and 299.9 ± 24.63mg of Quercetin equivalent wt/ g of extract respectively. The extract was showed powerful hydroxyl radical scavenging and lipid peroxidation activity (IC_{50} values of 364.78µg/ml, 124.40µg/ml, 358.09µg/ml) respectively. The current study revealed that HEAC fruits have significant free radical scavenging activity. **Conclusion:** Based on our outcomes, it was initiated HEAC fruits can act as a source of a beneficial drug due to presence of various active phytoconstituents, and extract showed potential antioxidant activity. These properties further act as supportive for preventing several diseases triggered by oxidative stress.

Keywords: *Anamirta cocculus*, hydroalcohol, phytochemicals, antioxidant, flavonoids

Introduction

Traditional herbal medicines are relatively safer than synthetic or chemically developed drugs. The traditional medicinal performance is very precious for the object that comes from thousands of years of experimental and blunder. Plant-based traditional knowledge has become an organized tool in the exploration for new sources of drugs and innovative chemical entity thus producing the basis of recent medicine and therapeutics (Raji et al, 2014). According to the World Health Organization, about 80% of the world's rural population currently relies on medicinal plants as their complementary or alternative source of health care improved efficacy (Chan, 2003).

Nowadays the use of herbal plants is growing all over the world for the treatment of various diseases, due to their potential antioxidant activities. It is generally understood that oxidative stress generates a large number of reactive oxygen species / free radicals, which play a significant role in the pathophysiology of many diseases, including inflammatory disorders. Oxidative stress is produced when there is inequality among the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the number of cellular antioxidants that can lead to multiple reactions causing damage or death of cells (Shebis et al., 2013). An imbalance between the body's antioxidant mechanisms and the generation of oxidative stress results in the development of chronic diseases such as autoimmunity such as rheumatoid arthritis, cell aging, and cancer. (Demirbag et al, 2005; Ozgocmen et al, 2006). The curative value of these plants has

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some active elements that produce a confident pharmacological exploit on populations. The most important property of this bioactive principle source of plants is that they are more effective with slight or no side effects when compared to the commonly used synthetic chemotherapeutic agents. The plants' phytochemicals are alkaloids, tannins, terpenoids and saponins, flavonoids, and phenolic compounds (Dhandapani and Sabna, 2008). These active constituents are used for the treatment of several diseases. These free radicals can be scavenged by the natural and synthetic antioxidant, but antioxidants from natural origin have attracted special interest.

Anamirta cocculus is a Southeast Asian and Indian climbing plant, Family: Menispermaceae, in English: Fish berry, Kannada: Kakamari. Picrotoxin toxic alkaloid with stimulating features is found in its fruit. The plant is larger stemmed the bark is "corky grey" with white wood. The fruit produced is a drupe, about 1 cm in diameter when dry (Wilma and Dichso, 2012).

The plant furnishing them is a strong, large, woody climbing shrub, large-stemmed about 10 cm in thickness timber. The leaves are shining, and spherical, fruits consist of dry, light, roundish nut, nearly ½ inch in diameter, greyish black color. Flowers greenish in lengthy panicles, drupes fruits, shaped kidney. Seeds are Globus and deeply excavated at the hilum. Distribution Western Ghats of South India (Nandakarni, 1982; Orient, 1996).

The fruit contains the harmless alkaloids paramenispermine and menispermine. Four quaternary alkaloids are berberine, palmatine, magnoflorine, stephorine, isoquinoline, columbarine, and tertiary alkaloid. The husk of *Anamirta cocculus (*AC) grains also contains non-poisonous, and also contains a yellow resin, fat, hypopicrotoxic acid. The seeds contain resin, free fatty acid, principally stearic acid, very poisonous, picrotoxin ($C_{30}H_{34}O_{13}$), and picrotoxic acid, it is escorted by the crystallisable, tasteless cocculin or anamirtin ($C_{19}H_{26}O_{10}$) crystallisable, insoluble in alcohol and ether. Levant berry is a current conventional remedy in Asia then adjacent regions, the infusion of the roots of HEAC is used to treat fevers, dyspepsia, and menstrual problems, headache, stomach ache. The fruit was used in very small doses to treat eruptive fevers and the powdered fruit was used to treat acute barbiturate poisoning, cure skin diseases. The seeds are destroyed head lice, fresh bark is useful for the snake bite (Basu and Kirtikar, 1991). The fruit juice is useful for ulcers and scabies. Picrotoxin has been used intravenously as an antidote against poisoning by barbiturates and morphine (Thomson, 2000). Very minute doses of Picrotoxin were used as a nerve tonic in schizophrenia, epilepsy, travel sickness, and giddiness. In homeopathic medicine, the drug is used for nervous exhaustion, attacks of dizziness, cramps, paralysis (Thomson, 2000). According to the Unani system of medicine, the fruit is slightly bitter, removes gas from the intestine, is well for rheumatism, and as an application for inflammations and treat cancer. (Jijith et al, 2016a).

Literature survey reveals that HEAC has been reported to possess the following Pharmacological activities of Antiulcer(Satya and Paridhavi, 2012), Anti-bacterial(Umer et al, 2015), Wound healing (Satya and Paridhavi, 2013), Larvicidal and adulticidal (Umer, 2014), Anthelmintics (Umer and Paul, 2013), Analgesic (Mohammad et al, 2010), Gross behavioral effects (Jijith et al, 2016b), Cardiovascular system (Jijith et al, 2016c), Anti-inflammatory (Satya and Paridhavi, 2016).

Preliminary phytochemical screening of plants is also required for the revolution and development of novel therapeutic agents with improved efficiency. The present study aimed to evaluate Physico-chemical properties, phytochemical, qualitative, quantitative, and antioxidant activity as well as the investigating from the fruit of AC and its hydroalcohol extract.

Materials and methods

Chemicals

Folin–Ciocalteu phenol mixture, quercetin, Gallic acid, anhydrous sodium carbonate, deionized water, methanol, petroleum ether, chloroform, benzene, ethanol, sodium nitrite, sodium hydroxide, aluminum trichloride, and all further chemical of laboratory-grade were used.

Plant material

The plant Fruits of *Anamirta cocculus* were collected from distributors from Gadgil Vanaushadi Store Belgaum, Karnataka, India. It was identified and authenticated by Professor S.A. Kappali, Department of Botany, Basaveshwar Science College, Bagalkot, and Karnataka.

Preparation of extract

The collected AC Fruits were gently washed in tap water treated with $KMNO_4$ solution, cleaned and removed seeds from fruits, completely dried under room temperature for 4 weeks protected from sunlight. The seed is removed from fruits of AC were dried under the shade then subjected to coarse milling and gated powder screened through a sieve # 44 to get uniform sized powder. The collected powder was subject to soxhlet extraction with petroleum ether to defat and then defatted fruits powder was macerated with hydroalcohol solvent water: ethanol (70%: 30%) for about 48 hours with occasional shaking. Macerate was decanted and filtered through muslin cloth and then through filter paper to obtain a clear extract (Mithra et al, 2012). This procedure was continual with an equal volume of hydroalcohol mixture. Macerates were collected in trays and vaporized to dryness at 31-35°C followed by completely dried and stored in an air-tight container and

placed in a refrigerator. The acquired dried extract was subjected to quantitative, qualitative, and phytochemical analysis and antioxidant activity.

Evaluation of physicochemical properties of powder of *Anamirta cocculus* (AC) fruits.

In the physico-chemical evaluation, such as ash value, total ash, acid insoluble ash, water-soluble ash, extractive values, alcohol soluble extractive value, water-soluble extractive, petroleum ether soluble extractive value, and loss on drying (LOD) were determined (Kokate, 2003; Anonymous,1996) The ash values represent the inorganic salts present in the drug. The extract obtained by the exhausting crude drug is indicative of approximate measures of certain chemical compounds they contain, the diversity in chemical nature, and the properties of contents of the drug.

Total ash value determination: 2 g of powder of AC fruits was reticent in a tared silica crucible at a temperature, not more than 450°C until able from ash was obtained. The subsequent ash was cool and weighed. The % of ash was deliberate regarding the crude drug.

Acid insoluble ash (AIS) value: The total ash found from 2 g of AC fruits ash was boiled for 5 min with dilute HCL of 25 ml and insoluble matter was composed an ash less filter paper. It was washed away with boiled water, burned, and evaluated. The % of acid-insoluble ash was planned with position to the crude drug.

Water-soluble ash value: Total ash obtained from 2 g of AC fruits ash was boiled for 5 min with water of 25 ml and the unsolvable material was composed of an ash less filter paper. It was washed away with hot water, ignited to a constant weight. The water-soluble ash was designed regarding the crude drug.

Alcohol soluble extractive value determination: Exactly weighed powder 5 g of AC fruits were taken and soaked with 100 ml of alcohol 95% for 24 hrs in an airtight vessel, the content was regularly shaken through the first 6 hrs and allowed for 18 hrs after 24 hrs the extract was filtered through filter paper and filtrate were evaporated finally the source was dehydrated at 105°C to constant mass and extractive value was calculated as a percentage (w/w) through reference to the crude drug.

Water-soluble extractive value determination: Exactly weighed (5 g) powder of AC fruits was exposed maceration with 100 ml of water in an airtight container for 24 hrs the content was regularly shaken for the period of first 6 hrs and permissible to remain for 18 hrs. After 24 hrs the extract was filtered through filter paper and filtrate were evaporated finally the extract were dehydrated at 105°C to a constant mass of extractive value were calculated as a percentage (w/w) using the reference to the dried crude drug.

Petroleum ether soluble extractive (PESE) value determination: Accurately weighed 5 g powder of AC fruits was subjected to maceration with petroleum ether of 100 ml in an airtight container for 24 hrs the content was frequently shaking the first 6 hrs and permissible to remain for 18hrs, After 24 hrs the extract was filtered through filter paper and filtrate were evaporated finally the extract were dehydrated at 105°C to a constant weight of extractive value was calculated as a percentage (w/w) with air-dried drug as reference.

Loss on drying (LOD): Loss on drying was the hurt in mass in percentage, resultant after volatile matter and water of some gentle that can be absorbed off under specified conditions. Weighed exactly 1.5 g of the processed AC fruits drug was placed in a porcelain tared dish and dehydrated at 105˚C in a burning air furnace to obtain endless mass and at that time weighed. From the modification in weight, % loss on aeration with to the dried crude drug as reference (Mallappa et al., 2019).

Qualitative phytochemical analysis of hydroalcohol extract of *Anamirta cocculus* (HEAC*)* fruits.

Accurately weighed 2 g of the HEAC was dissolved in 200 ml of own mother solvents to obtain a stock of concentration 1% (w/v). The extract was subjected to preliminary phytochemical screening by using different phytochemical analysis methods (Kokate, 2003; Harborne, 1998). Major constituents analysed were carbohydrates, Proteins, Amino acids, Glycosides, Alkaloids, Phytosteroids, Flavonoids, Saponins, tannins, and phenolic compounds.

Quantitative phytochemical analysis of hydroalcohol extract of *Anamirta cocculus* (HEAC) fruits

Total phenolic content determination: TPC was assessed by Folin Ciocalteu's manner. 1 ml of aliquots and standard gallic acid(GA) [6.25, 12.5, 25, 50, 100, and 200 µg/ml] was sited into the test tubes this was followed by the addition of 5 ml of distilled water and Folin Ciocalteu's reagent of 0.5 ml was mixed and surprized. After 5 min, 20 % sodium carbonate 1.5 ml was added and volume ended up to 10 ml with distilled water. It was permitted to incubate for 2 hr at room temperature (22 ^{0}C). The extreme blue color was developed. After incubation, absorbance was measured at 750 nm by using a UV-Visible spectrophotometer device. The extract was performed in triplicates. The blank was made using a reagent blank by solvent. Gallic acid(GA) was used as standard. The calibration curve was designed using standard GA. The data for total phenolic contents of HEAC fruit was expressed as mg of GA comparable wt/ 1 g of dry mass (Bhalodia et al., 2011; Patel et al., 2010).

Total flavonoid content determination: TFC was measured by colorimetric assay with aluminum chloride (Al Cl_3). 1 ml of aliquots and 1 ml standard Quercetin

solutions [6.25,12.5, 25, 50, 100, 200, 400 and 800 µg/ml] were situated into test tubes followed by the addition of 4ml of purified water, 0.3 ml of sodium nitrite (5 %) solution was added into each, after 5 min, 0.3 ml of Al Cl_3 10 % was mixed. Later 6 min. 2 ml of 1 M sodium hydroxide was added. Concluding, volume was making up to 10 ml with purified water and shaken well. An orange yellowish color was developed. This color solution was measured absorbance at 510 nm by using UV-visible spectrophotometer instrument, the distilled water was used as blank, Quercetin was used as standard. The sample extract was done in triplicates. The correction curve plotted against standard Quercetin, the data of total flavonoid of extract expressed as mg of Quercetin equivalents wt / 1 g of extract (Pallab et al, 2013).

***In-vitro* antioxidant activity of HEAC fruits:**

The DPPH method is described as a modest, rapid, and appropriate technique independent of sample separation for screening of many natural sources to radicals scavenging and to invention out promising candidates for commercial sense. Using the stable radical DPPH, the free radical scavenging activity was assessed in terms of hydrogen donating or radical scavenging ability (Sreejayan and Rao, 1996). 0.1 mM of DPPH in methanol solution was prepared and one (1) ml of this solution added with 3.0 ml of control [without the test compound, but an equivalent amount of methanol] and test solution at different concentrations of HEAC (3.9 to 500 µg/ml in methanol) in different test tubes, after 30 min absorbance was measured at 517 nm. The decrease in absorbance indicates increased RS activity was calculated by the formula. The maximum percentage inhibition of DPPH radical and IC_{50} values was determined. Absorbance was converted to DPPH radical-scavenging rate according to the equation (Ganapaty et al, 2007).

DPPH RS activity percentage = {(Abs of control – Abs of sample) / Abs of Control} x 100

***In vitro* hydroxyl radical scavenging activity of HEAC fruits:**

Hydroxyl radical scavenging action was resolute according to the method of Klein et al. various concentrations (31.25 to 1000 µg/ml) of hydroalcohol extract of *Anamirta cocculus* (HEAC) extract were taken in different test tubes and evaporated to dryness. 1 ml of EDTA iron solution (ferrous ammonium sulfate0.13%and EDTA0.26%), 0.5ml of EDTA (0.018%), and 1ml of DMSO (0.85 % v/v in 0.1M phosphate buffer, PH 7.40) were added to these tubes and the reaction was initiated by adding 0.5ml of 0.22% ascorbic acid, HEAC extract containing test tube were capped tightly and heated on water bath at 80-90°C for 15 min the reaction was terminated by addition of 1 ml ice-cold TCA (17.5% W/V), 3 ml Nash reagent (75.0 g ammonium acetate, 3 ml glacial acetic acid, and 2 ml acetyl acetone were mixed and raised to one litre with distilled water) was added to all the HEAC extract containing test tubes and left at room temperature for 15 min for color development. The intensity of yellow color formed was measured spectrophotometrically at 412 nm against reagent blank (Singh et al., 2002; Kleinet al., 1991). Data are shown in table 5 and figure 5, 6. Maximum proportion inhibition of hydroxyl radical scavenging is calculated by the formula.

Percentage Hydroxyl radical scavenging activity = $(A_{control} - A_{sample} / A_{control}) \times 100$

***In vitro* lipid peroxidation activity of HEAC fruits**

The point of lipid peroxidation was evaluated by assessing the TBARS (Thiobarbituric acid reactive substances). Briefly, different concentrations of hydroalcohol extract of *Anamirta cocculus* (15.62 to 1000 µg/ml) were added to the liver homogenate. Lipid peroxidation was initiated by adding 100 µl of $FeSO_4$ solutions 15 mmol to liver homogenate 3 ml. After 30 min, 100 µl of this reaction mixture was placed in a test tube containing 1.5 ml of 10% trichloroacetic acid (TCA) and centrifuged after 10 min. the supernatant was separated and mixed with 1.5 ml of 0.67% thiobarbituric acid (TBA) in 50% acetic acid. The combination was heated in a water-bath at 85°C for 30 min to complete the reaction. The intensity of the pink-colored complex was measured at 535 nm with a spectrophotometer (Madan et al., 2005; Okhawa et al., 1979) Data are shown in table 6 and figure 7, 8. Maximum percentage inhibition of lipid peroxidation is calculated by the formula.

Results

Physicochemical properties of AC fruits were found Ash value percentage 10.54 ± 0.57 are imperative quantitative parameters describing transparency of plant crude drug. The Water-soluble ash value percentage 9.76± 0.13 of AC denotes that powder has more solubility in water. The mean percentage of water, alcohol, and petroleum soluble extractive values were 23 ± 0.11, 19 ± 0.08, and 7.9 ± 0.31 respectively. The extractive value of the crude drug in a certain solvent is an index for testing the purity of the crude drug. Results are summarized in table 1.

Preliminary phytochemical screening

Table 1. Physicochemical properties of *Anamirta cocculus* fruits

Parameters	Values in %(w/w)
Ash Value	
Total ash value	10.54 ± 0.57
Acid insoluble ash value	3.8± 0.23
Water-soluble ash value	9.76± 0.13
Extractive value	
Alcohol soluble extractive value	23.54± 0.11
Water-soluble extractive value	19.65± 0.08
Petroleum ether soluble extractive value	7.9± 0.31
Loss on drying	1.8± 0.06

Primary phytochemical screening of HEAC fruits discovered that the presence of carbohydrates, proteins, glycosides, alkaloids, steroids, flavonoids, saponins, phenolic and tannins compounds were present in the extract and the results are summarised in Table 2.

Total phenolic and flavonoid matters

Phenolic elements are significant plant ingredients having antioxidant activity due to their redox characteristics. As a basis quantitative determination, flavonoid contents in selected plant extract were determined using aluminium chloride in a colorimetric method. The total phenolic contents in the HEAC fruits were found to be 16.16 ± 0.085 mg of GA equivalent wt/g of extract. The concentration of flavonoids in plant HEAC fruits was found to be 299.9 ± 24.63 mg of Quercetin equivalent wt. / g of extract. Results were potted in table 3 and Figure 1 and 2.

***In-vitro* antioxidant activity**

Concentrations of flashing from 3.94 to 500 µg / ml of HEAC fruits tested for antioxidant action by DPPH radical scavenging activity. The maximum percentage inhibition

Table 2. Qualitative phytochemical analysis of hydroalcohol extracts of *Anamirta cocculus* (HEAC) fruits

Phytochemical constituents	Name of the test	Observation	HAAC
Carbohydrates	Molish's test	A reddish-violet ring at the junction between two liquids	Positive
	Fehling's test	Brick red precipitate	Positive
Proteins and Amino acids	Biuret test	Purple color	Positive
	Millon's test	No red precipitate	Negative
	Ninhydrin test	Pink color	Positive
Glycosides	Legal's test	Red color	Positive
	Borntrager's test	Color change	Positive
Alkaloids	Mayer's test	White yellow precipitate	Positive
	Dragendroff's test	Orange-red precipitate	Positive
	Hager's test	Yellow color precipitate	Positive
	Wagner's test	Reddish-brown precipitate	Positive
Steroids	Salkowski test	Formation of brown ring	Positive
	Liebermnn-Burchard test	Bluish-green color	Positive
Flavonoids	Shinoda test	pink color	Positive
	Ferric Chloride test	Intense green color	Positive
	Alkaline reagent test	Yellow to colorless	Positive
	Lead Acetate solution test	Yellow precipitate	Positive
Saponins	Foam test	Formation of foam	Positive
Tannins and Phenolic Compounds	5% FeCl3 solution	Deep blue black color	Positive
	Lead acetate solution	White precipitate	Positive
	Dilute HNO3	No reddish to yellow color	Negative

Positive: Indicates the presence of phytochemicals: **Negative**: Indicates absence of Phytochemical

Table 3. Total phenolic and flavonoids content of HEAC fruits

Extract	Phenolic content (mg of Gallic acid equivalent weight/ g of extract)	Flavonoids content (mg of quercetin equivalent wt/ g of extract)
HEAC	16.16 ± 0.085	299.9 ± 24.63

All values are expressed in Mean ± SEM;. HEAC = Hydroalcohol extract of *Anamirta cocculus.*

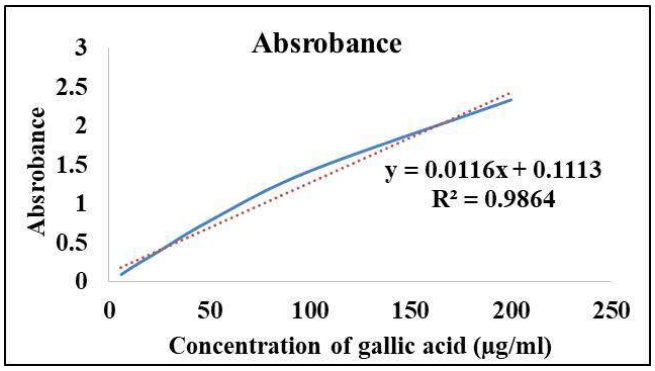

Figure 1. Total phenolic content for standard Gallic acid.

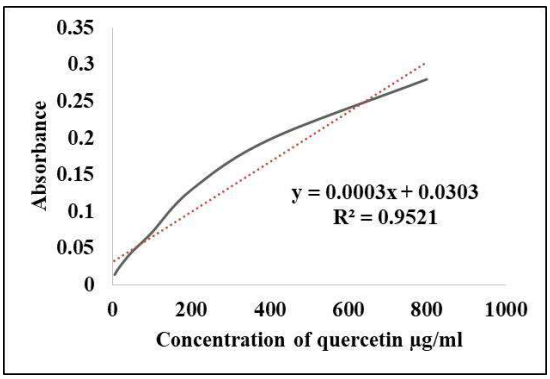

Figure 2. Total flavonoid content for standard Quercetin

FRS activity with different concentrations of the extract is given in Table 4. The DPPH RS percentage is shown in figures 3 and 4. A significant decrease in the concentration of DPPH radicals scavenging ability of HEAC fruits. The maximum scavenging activity of Ascorbic acid as standard and HEAC fruits exerted an inhibition at 500 µg/ml of 97.01 % and 86.19 % respectively and the IC_{50} (the inhibitory concentration at which there is 50% reduction of free radical) value of ascorbic acid and extract was found 58.31µg /ml and 124.4 µg /ml respectively.

The hydroxyl radical scavenging activity is shown in figures 5, 6 and table 5. Quercetin and hydroalcohol extract of *Anamirta cocculus* exerted an inhibition of 97.03% and 82.69 % at 1000 µg/ml and the IC_{50} of the Quercetin and extract was 178.71 µg/ml and 364.78 µg/ml. respectively similarly.

The lipid peroxidation activity is shown in figures 7, 8 and table 6. Quercetin and hydroalcohol extract of *Anamirta cocculus* exerted an inhibition of 98.01 % and 84.66 % at 1000 µg/ml and the IC_{50} of the Quercetin and extract was 197.93 µg/ml and 358.09 µg/ml respectively.

Table 4. DPPH radical scavenging activity

Concentration of solution (µg/ml)	Percentage (%)		IC_{50} Value (µg/ml)	
	Ascorbic acid	HEAC	Ascorbic acid	HEAC
3.9	32.14	23.64	58.31	124.4
7.81	38.64	31.25		
15.65	45.06	39.58		
31.25	49.87	45.89		
62.5	56.54	49.87		
125	65.25	54.21		
250	78.32	69.45		
500	97.01	86.19		

HEAC= Hydroalcohol extract of *Anamirta cocculus*

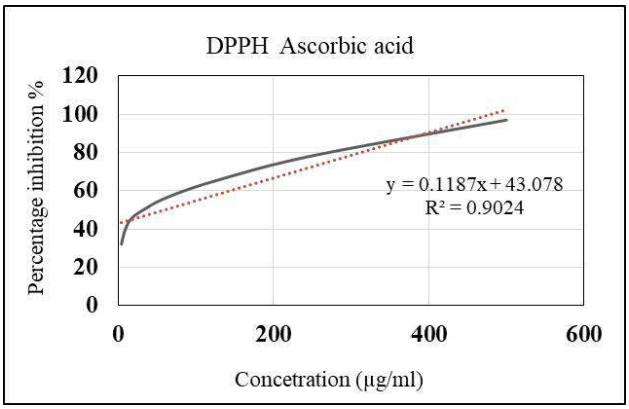

Figure 3. DPPH radical scavenging (RS) activity of Ascorbic acid.

Table 5. *In vitro* hydroxyl radical scavenging activity

Concentration of solution µg/ml	Percentage (%)		IC_{50} Value (µg/ml)	
	Quercetin	HEAC	Quercetin	HEAC
15.62	24.01	15.49	197.93	358.09
31.25	35.67	21.51		
62.5	44.12	37.45		
125	49.1	45.09		
250	62.26	49.43		
500	77.21	63.87		
1000	98.01	84.66		

HEAC= Hydroalcohol extract of *Anamirta cocculus*

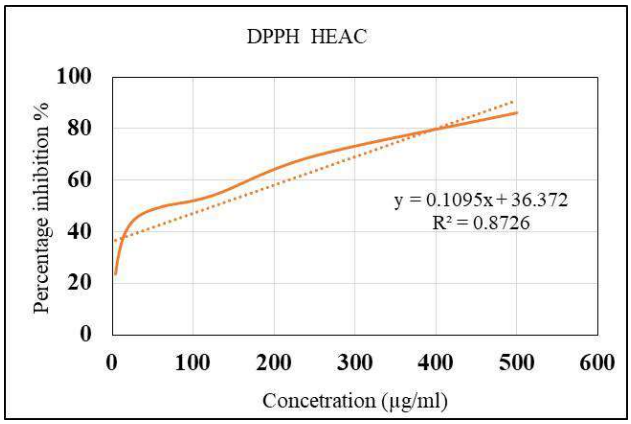

Figure 4. DPPH radical scavenging (RS) activity of HEAC fruits.

Table 6. *In vitro* lipid peroxidation activity

Concentration of solution µg/ml	Percentage (%)		IC_{50} Value (µg/ml)	
	Quercetin	HEAC	Quercetin	HEAC
31.25	31.02	21.65	178.71	364.78
62.5	39.45	33.54		
125	47.12	37.36		
250	64.26	49.58		
500	79.25	62.55		
1000	97.03	82.69		

HEAC= Hydroalcohol extract of *Anamirta cocculus*

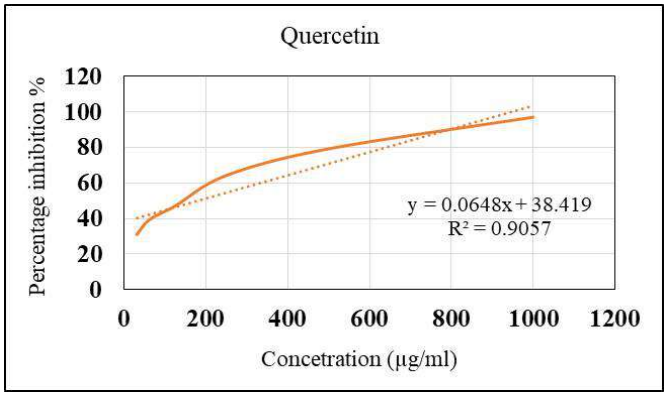

Figure 5. *In vitro* hydroxyl radical scavenging activity of Quercetin

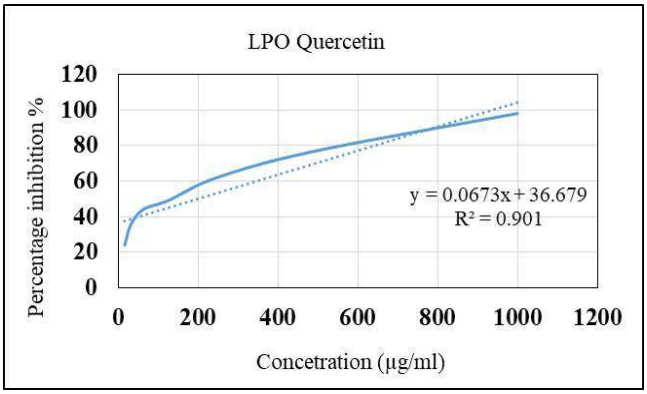

Figure 7. *In vitro* lipid peroxidation activity of Quercetin

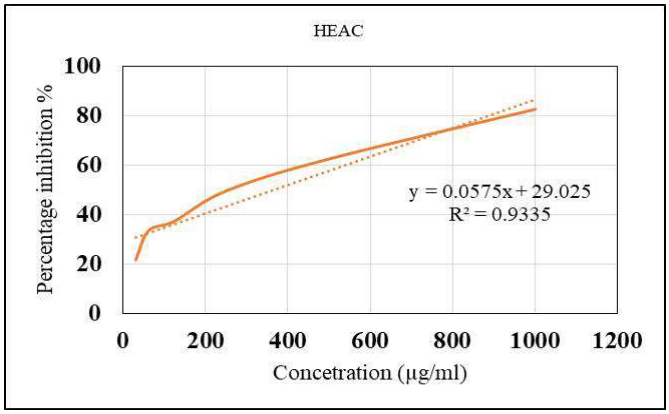

Figure 6. *In vitro* hydroxyl radical scavenging activity of HEAC

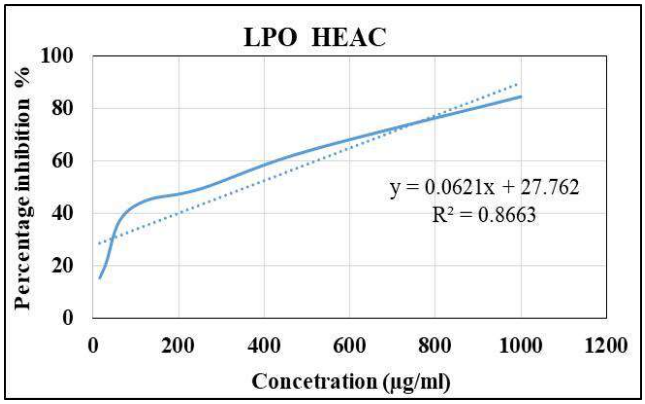

Figure 8. *In vitro* lipid peroxidation activity of HEAC

Discussion

The physical constant evaluation of the drugs is an important parameter in detecting adulteration or inadequate handling of drugs. Physicochemical characterization of powder of studied *Anamirta cocculus* fruit is shown in table 1. The fruit showed less moisture content, the moisture content of the crude drug was found below the moisture content of drugs could be at a minimal level to depress the growth of bacteria, fungi, or yeast during storage. The moisture content of the drug discloses the stability and its shelf-life. High moisture content can unfavourably affect the active ingredient of the drug. As a result, minimal moisture content can provide optimal stability and shelf life. It can serve as a valuable source of information and provide appropriate standards to establish the quality of this plant material in future revision or application. The ash value was determined by 3 different forms viz., total ash, acid insoluble ash, and water-soluble ash. The total ash, acid-insoluble ash, water-soluble ash, was found to be respectively. The ash value of a crude medication is used to determine its quality and purity. It indicates the presence of various impurities like carbonate, silicate, and oxalate. The water-soluble ash is used to estimate the amount of inorganic complex present in the drug. The acid-insoluble ash measures the amount of silica present, especially pebbles and it indicates contamination with earthy material. The water-soluble percentage of total ash is called water-soluble ash. Less amount of these 3 parameters indicate that the inorganic matter and silica were less in the fruit of *Anamirta cocculus*.

The alcohol-soluble extractive value, water-soluble extractive value, and chloroform soluble extractive value were suggestive for the same determination. The water-soluble extractive value and alcohol-soluble extractive value was higher than the Chloroform soluble extractive value in this study, and it was found to be respectively, this shows that the constituents of the drug are more extracted and soluble in water, alcohol as compared to Chloroform. The extractive values are important for determining the chemical constituents present in the crude medicine, as well as estimating the soluble elements in a given solvent (Rakholiya et al., 2016 ; Kaneria and Chanda, 2011).

Medicinal herbs have long been used to treat a variety of human illnesses. Phytochemical analysis of plants revealed the presence of constituents that are known to exhibit medicinal as well as action on the human body (Vaghasiya et

al., 2008). Phytochemical analysis shows many medicinally important secondary metabolite kinds of phytoconstituents, such as alkaloids, cardiac glycosides, saponins, and triterpenes, indicate that the plant has a high profile value and can be exploited to treat various kinds of diseases. Because these secondary metabolites are present, the plant may have medicinal use. The results of qualitative phytochemical analysis of the HEAC fruit contains phytoconstituents like carbohydrates, alkaloids, flavonoids, glycosides, saponins, steroids, Tannins and Phenolic compounds are shown in table 2.

Phenolic compounds are of great importance as they have high antioxidant potential which protects the human body from oxidative stress, which may lead to diseases including cancer, cardiovascular problems, and aging. Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoids, phenolic acids, etc (Ali et al., 2008). Flavonoids and tannins are two main antioxidants and free radical scavengers. Flavonoids are water-soluble phytochemicals that reduce free radicals by quenching, up-regulating, or protecting antioxidant defences and chelating radical intermediate compounds(Ndhlala et al., 2010).

At normal temperature, DPPH is a stable free radical that accepts an electron or hydrogen radical to form a stable diamagnetic molecule. The ability of DPPH radicals to reduce was assessed by the decrease in their absorbance at 517 nm caused by antioxidants. The scavenging activity of HEAC fruits is responsible for the considerable decrease in DPPH radical concentration. Hydroxyl radicals are the main active oxygen species triggering lipid peroxidation and massive biological injury. The IC_{50} value indicates that the plant extract of HEAC is a better hydroxyl scavenger than the standard Quercetin. Significant activity in scavenging hydroxyl radicals. Lipid peroxidation denotes to the oxidative degradation of lipids. It is the method in which free radicals bargain electrons from the lipids in cell membrane, resultant in cell damage. This process continues by a free radical chain reaction tool. It most often affects polyunsaturated fatty acids. If not finished fast sufficient, there will be destruction to the cell membrane, which consists mainly of lipids. In addition, end-products of lipid peroxidation may be mutagenic and carcinogenic(Chandra Mohan et al., 2012). The IC_{50} value indicates that the plant extract of HEAC is a better lipid peroxidation than the standard Quercetin.

Conclusion

The present study concluded that the *Anamirta cocculus* fruit has the potential to act as a source of useful chemical constituents due to the presence of various phytochemical constituents such as carbohydrates, alkaloids, flavonoids, glycosides, saponins, steroids, tannins, and phenolic compounds. These phytoconstituents look as potential to act as a source of useful constituents and to progress the health grade of the users as a result of the presence of several compounds for good health. HEAC also contains sufficient amount of phenolic and flavonoid compounds and these compounds contribute to the antioxidant activity of HEAC. Which will help prevent various diseases caused by oxidative stress.

Conflict of interest:

The authors have No conflicts of interest regarding this exploration**.**

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References

Ali SS, Naresh K, Abhinav L, Angad S, Hallihosur S, Abhishek S, Utpal B. 2008. Indian medicinal herbs as a source of antioxidants. Food Research International, 41:1-15.

Anonymous, Indian Pharmacopoeia, Vol. II, 4th ed, The Controller of Publications, Ministry of Health and Family Welfare, Government of India, New Delhi, 1996;53-54.

Basu BD, Kirtikar KR, and Basu K. 1991. Indian Medicinal Plants, Bishen Singh Mahendra Pal Singh. 2nd ed: 80-83.

Chan K. 2003. Some aspects of toxic contaminants in herbal medicines. Chemosphere, 52 (9):1361-71.

Chandra SM, Balamurugan V, Elayaraja R, Prabakaran AS. 2012. Antioxidant and phytochemical potential of medicinal plant *kalanchoe pinnata. International journal of pharmaceutical sciences and Research,* 3(3):881-885.

Demirbag R, Yilmaz R, Erel O, Gultekin U, Asci D. 2005. The relationship between the potency of oxidative stress and severity of dilated cardiomyopathy. Canadian Journal of Cardiology, 21:851-588.

Dhandapani R, Sabna B. 2008. Phytochemical constituents of some Indian medicinal plants. Ancient Science Journal of Life, 27(4):1–8.

Ganapaty S, Chandrashekhar VM, Chitme HR, Lakashmi MN. 2007. Free radical scavenging activity of *gossypin and nevedensin*: An in-vitro evaluation. Indian Journal Pharmacology, 39(6):281-83.

Harborne JB. 1998. Phytochemical methods: A guide to modern techniques of plant analysis. 3rd ed. Chapman and Hall Int. ed., New York.

Jijith US, Sudhakaran Nair CR, Pramod K. 2016. Acute toxicity and gross behavioural effects of alcoholic extract of *Anamirta cocculus* wild. *Research Journal of Pharmacology* and Pharmacodynamics 8(1):01-04.

Jijith US, Sudhakaran Nair CR, Pramod K. 2016. Unveiling

the effect of a stem bark extract on the cardiovascular system. Indo American Journal of Pharmaceutical Sciences, 3(5):406-09.

Jijith US, Sudhakaran N, Ajithkumar KC, Pramod K. 2016. Phytochemistry and Pharmacology of *Anamirta cocculus* Willd. *Research Journal* of *Pharmacognosy and Phytochemistry*, 8(2):90-92.

Kaneria M, Chanda S. 2011. Phytochemical and pharmacognostic evaluation of leaves of *Psidiumguajava* L. Pharmacognosy Journal, 3(23):41-5.

Klein SM, Cohen G, Cederbaum AJ. 1991. Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical generating system. Biochemistry, 20:6006-12.

Kokate CK. 2003. Practical Pharmacognosy. 4th ed, Vallabh Prakashan, New Delhi, 122-126.

Madan Mohan P, Raghavan G, Ajay Kumar Sr, Palpu P. 2005. Free radical scavenging potential of *Saussarea costus*. Acta Pharmceutica, 55:297-304.

Mallappa HS, Chandrashekhar VM, Iranna SM, Basavaraj H. 2019. Qualitative and quantitative phytochemical analysis of seeds. Asian Journal of Pharmacy and Pharmacology, 5(2):290-297.

Mithra NH, Shishir S, Mahalaxmi Y, Amit P. 2012. Evaluation of Antimicrobial Activity of Aqueous and Hydro-Alcoholic *Curcuma Longa* Extracts against Endodontic Pathogens. IOSR Journal of Pharmacy, 2(2):192-198.

Mohammad HI, Iffat Zareen A, Mohammad TS, 2010. Analgesic Activity of *Anamirta cocculus* Linn. Southeast University Journal of Science & Engineering, 5(5):91-93.

Nayan RB, Pankaj BN, Acharya RN, Shukla VJ. 2011. Evaluation of *in vitro* antioxidant activity of flowers of *Cassia fistula* Linn. International Journal of PharmTech Research, 3(1):589-599.

Nandakarni AK. Indian materiamedica, Popular Prakashan1982. p. 99.

Ndhlala AR, Finnie JF, Johannes VS. 2010. In vitro antioxidant properties, HIV-1 reverse transcriptase, and acetyl cholinesterase inhibitory effects of traditional herbal preparations sold in South Africa. Molecules, 15:6888-6904.

Okhawa H, Ohishi N, Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95:351-55.

Orient longmanm. Indian medicinal plants a compendium of 500 species. 1996.1-143.

Ozgocmen S, Ozyurt H, Sogut S, Akyol O. 2006. Current concepts in the pathophysiology of fibromyalgia the potential role of oxidative stress and nitric oxide . Rheumatology International, 26:585-597.

Pallab K, Barman Tapan K, Pal TK, Kalita R. 2013. Estimation of total flavonoid content and antioxidant activities of methanolic whole plant extract of Biophytum sensitivum Linn. Journal of Drug Delivery & Therapeutics, 3(4):33-37.

Patel A, Patel NM. 2010. Estimation of flavonoid, polyphenolic content, and in vitro antioxidant capacity of leaves of Tephrosia purpurea Linn. International Journal of Pharmaceutical Science and Research, 1(1):66-77.

Raji M, Veeraiyan, Binu T, Chitrambalam M. 2014. An Ethnobotanical survey of medicinal plants used by kolli Malayalis of Namakkal district Eastern Ghats Tamil Nadu India. European Journal of Environmental Ecology,8(1): 33-43.

Rakholiya K, Mital K, Sumitra C. 2016. Physicochemical and Phytochemical Analysis of Different Parts of Indian Kesar Mango–A unique variety from Saurashtra Region of Gujarat. Pharmacognosy Journal, 8(5):502-06.

Satya V, Paridhavi M. 2016. Evaluation of anti-inflammatory activity of *Anamirta cocculus* Linn. Fruit. *International Journal* of *Pharmacy* and *Pharmaceutical Sciences*, 4(1):30-35.

Satya V, Paridhavi M. 2013. Wound healing activity of fruits of *Anamirta cocculus Linn* in rats. *International Journal* of *Pharmacy* and *Pharmaceutical Sciences*, 4(5):280-282.

SatyaV, Paridhavi M. 2012. Evaluation of anti-ulcer activity of detoxified pericarp extract of *Anamirta cocculus* fruit. Journal of Pharmaceutical Research, 5(12):5474-5480.

Shebis Y, Iluz D, Tahan YK, Dubimsky Z, Yehoshma Y. 2013. Natural antioxidants function and sources. Food Nutrient Science, 4:643-649.

Singh RP, Chidambra, Jayaprakasha 2002. Studies on the antioxidant activity of *pomegranate* (punicagranatum) peel and seed extracts using *in vitro* models. Journal of agricultural and food chemistry, 50:81-86.

Sreejayan N, Rao MN. 1996. Free radical scavenging activity of curcuminoids. Drug Research, 46:169-71.

Thomson PDR Staff. PDR for Herbal Medicines, Thomson PDR. 2nd ed; 2000: 312–313.

Trease GE, Evan WC. 2003. Textbook of Pharmacognosy, Bailliere Tindall. 15th ed: 320.

Umer Q, Paul VI, Ganesh P. 2015. Preliminary Phytochemical screening and *in vitro* antibacterial activity of *Anamirta cocculus* (linn.) seeds. –Journal of King Saud University Science, 2(27):97-104.

Umer Q, Paul VI. 2013. In vitro Nematicidal (Anthelmintic) Property of the Seed Extracts of Anamirta cocculus. L Against Pheretima Posthuma (L. Vaill.). *Ethiopian journal of applied sciences* and

technology, 4:65-75.

Umer Q. 2014. Bioefficacy of *Anamirta cocculus* L. seed extracts against dengue vector, *Aedesaegypti* Linn. Asian Pacific Journal of Tropical Disease 2(4): S556-62.

Vaghasiya Y, Nair R, Chanda S. 2008. Antibacterial and preliminary phytochemical and Physico-chemical analysis of *Eucalyptus citriodora* Hk leaf. Natural Product Research, 22(9):754-62.

Wilma C, Dichoso ED. 2012. Research information series On Ecosystems volume 12, No.2. Useful plant species with Toxic Substance. Department of Environment and Natural Resources, p. 9.