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Antimicrobial and Antioxidant Activity of Coffee Pulp Extract

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Abstract:
The aim of the present study is to determine antioxidant and antimicrobial activity of coffee pulp methanol extract. The antioxidant activities were evaluated by using two different methods that are Reducing power Assay and DPPH. The antimicrobial activity was done against five bacterial species of ***Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa*** *using agar well diffusion method. Coffee pulp extract shows higher reducing power and antioxidant activity in 250µl/ml concentration. Similarly, Antimicrobial activity of coffee pulp shows highest zone of inhibition against selected five bacterial pathogens.*

Key words: *pulp, antimicrobial, antioxidant, DPPH, Reducing power assay*

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I. Introduction:

Worldwide coffee is a popular beverage. Coffee (Coffee arabica) belongs to Rubiaceae family. The most famous species are *Coffee arabica L, (C. arabica), Coffee Robusta L, (C. Robusta)* **Tizian Klinge et al., (2020)**. The ideal climatic conditions to grow coffee are related to temperature and rain fall, temperature in the 73^0F (23^0C) and 82^0F (28^0C) with rainfall incidence in the range of 60-80 inches.

In India Karnataka and Andhra Pradesh are the most suitable area for Arabica coffee cultivation. In Andhra Pradesh Coffee is grown in the agency areas of Chintapalli, Paderu and Maredumilli of Visakhapatnam and East Godavari districts. The present study was aimed to investigate the Antimicrobial and Antioxidant Activity of coffee pulp methanolic Extract.

During the processing of coffee cherry to obtain coffee seed, coffee by products will generate (pulp, husk and silver skin). Coffee pulp and husk are the major by products, for every 2tons of coffee fruits1ton of coffee pulp was obtain. **Roussos S, de los Angeles Aquiahuatl M, del Refugio Trejo-Hernandez M, Gaime Perraud I, Favekla E, Ramakrishna M et al., (1995**). It is normally left decomposed naturally to become a fertilizer used in the coffee field. This causes a lot of environmental problems as a result of its slow decomposition process.

With regard to composition, it is known that it changes according to the coffee variety and the cultivation zone. It consists of proteins (9–11%), lipids (2–17%), cellulose (13–27%), tannins (4.5%), pectic materials (6.5%), reducing sugars (12.4%), non-nitrogenous materials (57–63%), and caffeine (1.3%) **(Pleissner et al., 2016)**. These findings shows that coffee pulp could be used as source of antioxidant and phenolic compounds. Its use in the food industry would solve an environmental problem, as well as proposing a novel source of bioactive compounds **(Stephania Rosales Delgado et al., 2019).** In the last years Hydroxy cinnamic acids (HAs) have been recognised as natural antioxidants. HAs like chlorogenic (ChA), caffeic (CA) and ferulic acid (FA) are natural compounds in coffee pulp (*1,2*). They have only one aromatic ring and several hydroxyl groups (*3,4*). FA, CA and ChA are HAs present in coffee pulp with higher antioxidant properties (**Miguel Angel Arellano-González et al., 2011)**.

II. Materials And Methods:

Collection of sample:

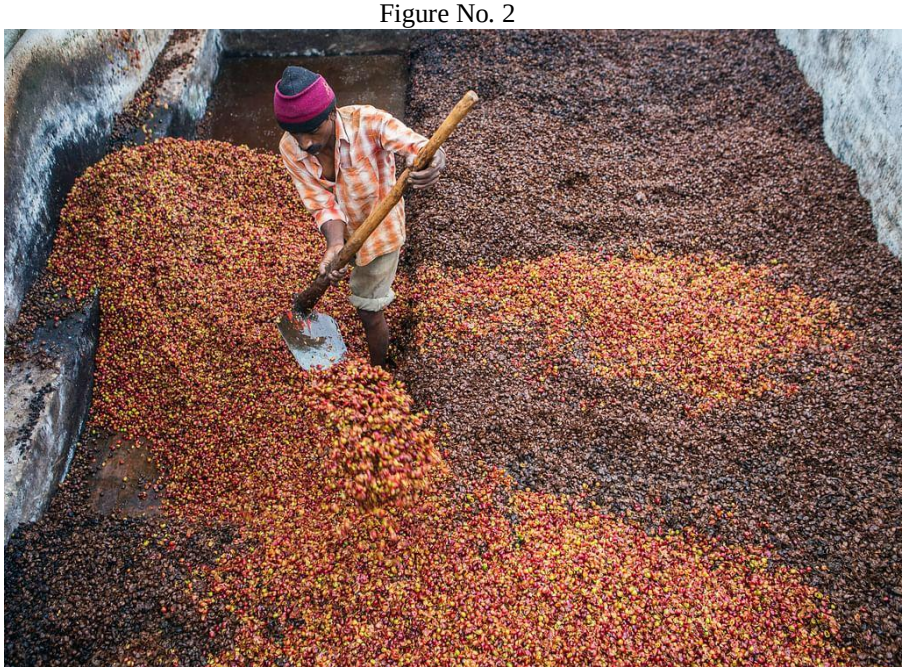
This study was carried out during the period of November 2019 to February 2020 in the coffee growing zone of the center R.V Nagar, G.K Veedhi Mandal, Visakhapatnam district, Andhra Pradesh.

R.V. nagar is surrounded by Chintapalle Mandal towards East, Koyyuru Mandal towards South, G. Madugula Mandal towards East, Golugonda Mandal towards South.

The harvested coffee cherries are brought to the processing mill to separate coffee seed from the fruit. During this process coffee by products (pulp, husk and silver skin) will generate. This waste was collected and transferred to laboratory with in 24hrs. The sample was ground with blender to make paste.

Figure No.1

Figure No. 2



Extraction:

The pulp sample (150gm) was extracted with 200ml of each solvent separately by using a Soxhlet extractor for 2 to 5h at a temperature not exceeding the boiling point of the solvent. The solvent methanol was used for the extraction. The extract was distilled by the distillation unit and were transferred amber glass bottles and kept at 4^{o}c. The extract was dissolved in Dimethyl sulphoxide (DMSO) to prepare different concentrations (50µl, 100µl,150µl 200µl).

Screening of antimicrobial activity:

Tested organisms:

Gram positive organisms ***Staphylococcus aureus (MTCC-3160), Streptococcus pyogenes (MTCC-2327) and*** Gram-negative organisms ***E.coli (MTCC-443), Pseudomonas aeruginosa, Klebsiella pneumonia (MTCC-452).***

Agar well diffusion method:

24hrs of fresh inoculum were spread on freshly made nutrient agar plates with sterile glass spreader. After, five wells of 8mm diameter bored on culture plates with borer, four wells for aliquots of pulp extract with different concentrates (50µl, 100µl, 150µl and 200µl) and one for the standard solution. Each well filled with 20µl pulp extract with different concentrations and the portion of standard solution was filled. The antibiotic (Erythromycin) used as standard. The plates were incubated at 37^{o}c for 24hrs.

Antioxidant activity:

DPPH radical scavenging activity method:

The scavenging activity of pulp extract was estimated according to the procedure described by **Shimada *et al.*, (1992)** with some modifications. Firstly, an aliquot of 0.5 ml of sample extracts at different concentrations were added to test tubes with 2.9 ml of 200 µM DPPH radical in ethanol. The mixture was shaken vigorously and left to stand for 30 min in the dark at room temperature. The reaction mixture was determined at 515 nm with UV-vis spectrophotometer. Extraction solvent was used as blank while mixture without extract served as control. Ascorbic acid was used as a standard. The scavenging effect was calculated based on the following equation:

Scavenging effect (%) = 1- [(Absorbance sample/ Absorbance control) x 100]

EC50 value (mg/ml) was defined as the total antioxidant needed to decrease the initial DPPH free radicals by 50%. It was determined from the plotted graph of scavenging activity against various concentrations of the sample extracts.

Reducing power

The reducing power of extracts was determined by the method of **Oyaizu et al., (1986).** 1 ml of extracts were mixed with 2.5 ml of phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixtures were incubated at 50^{0}C for 20 min. After that, trichloroacetic acid (10%, 2.5 ml) was added to the mixture and centrifuged. Finally, the upper layer was mixed with distilled water (2.5ml) and 0.5 ml of 0.1% ferric chloride ($FeCl_3$). The absorbance of the solution was measured at 700nm in spectrophotometer. Higher absorbance of the reaction mixture indicated that the reducing power is increased. Ascorbic acid and BHA were used as positive control.

Table 1: Scavenging Effect of Pulp Methanolic Extract

	50 µl	100 µl	150 µl	200 µl	250 µl
DPPH	44.2%	46.1%	46.5%	52.4%	52.6%
Reducing power Assay	0.09	0.14	0.18	0.22	0.27

Table 2: Antimicrobial Activity of Pulp Methanolic Extract

	Zone of inhibition in mm				
Micro organism	50µl	100 µl	150 µl	200 µl	Control
Streptococcus pyogenes	12mm	13mm	14mm	15mm	10mm
Staphylococcus aureus	15mm	16mm	16mm	20mm	11mm
E.Coli	22mm	23mm	23mm	26mm	12mm
Klebsiella Pneumoniae	15mm	17mm	18mm	18mm	10mm
Pseudomonas aeroginosa	13mm	13mm	15mm	16mm	10mm

Figure No 3 Anti microbial activity of pulp extract

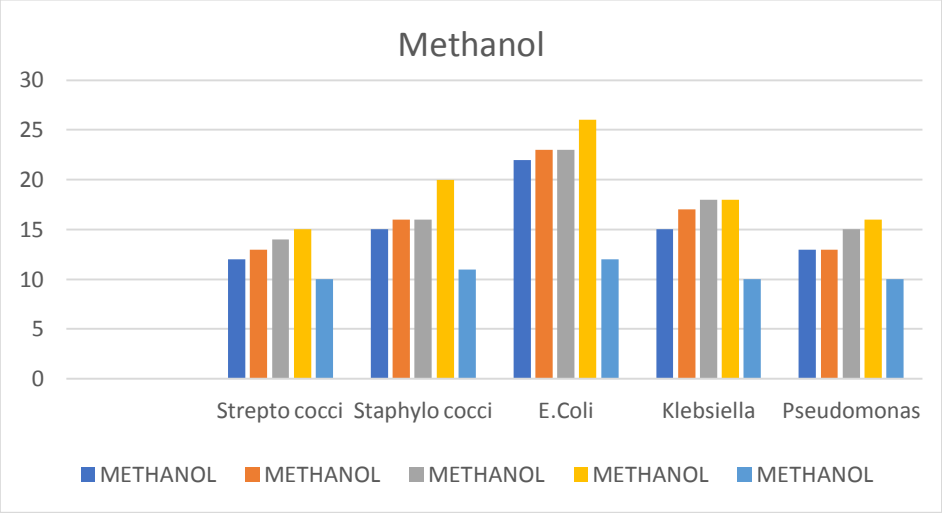
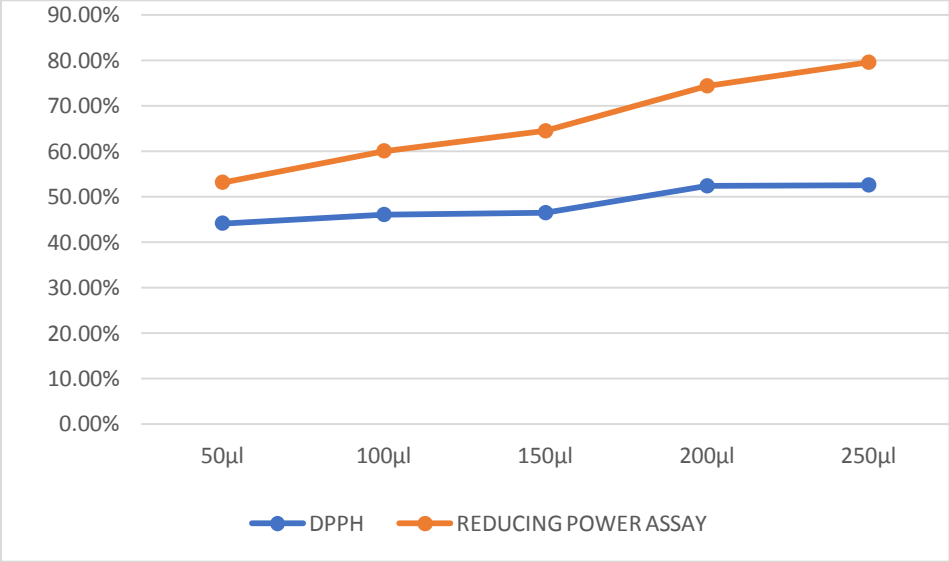


Figure No 4 Antioxidant Activity of methanol extract of Coffee Pulp



Methanol Extract of coffee pulp showing highest and lowest zone of inhibition against *E. Coli* and *Staphylococcus aureus*

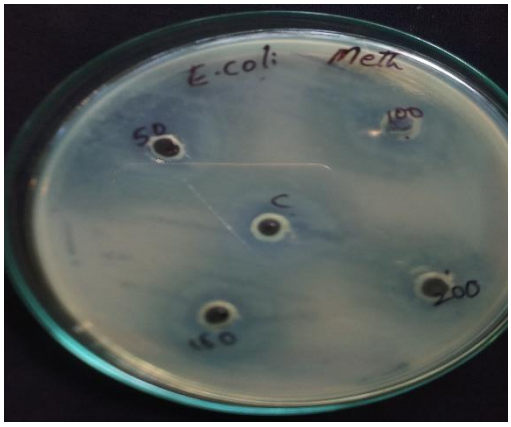


Fig no:5

Fig no:6

III. Results and Discussion:

The methanol extract of pulp was subjected to screening for possible antioxidant activity by the DPPH free radical scavenging method. Scavenging the stable DPPH radical is widely used method to evaluate the antioxidant activity in comparison to other method because this method is simple, requires short period of time and sensitive.

DPPH is a stable free radical that shows a characteristic absorbance at 517 nm, which decreases significantly when exposed to radical scavengers by providing hydrogen atom or electron to be a stable diamagnetic molecule. Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. Scavenging effects of methanol extracts from pulp on DPPH radical increased with the increased concentrations. According to the Table1, Coffee Pulp showed higher radical scavenging activity which was 52.6% in 250µg0/ ml concentration.

This study suggests that the antioxidant activity of methanolic extracts from pulp is due to phenolic compounds content. These results are supported by the evidence of many studies on antioxidant activity *in vitro* and *in vivo*, which show the action mechanism of flavonoids, including the ability to scavenger free radicals, chelate metal ions and inhibit oxidases. In addition, they can increase the availability of endogenous antioxidants, as well as the activity of antioxidant enzymes, and inhibitors of enzymes involved in the generation of ROS (radical oxygen species) (**Trueba, 2003**).

Reducing power of methanolic extract from pulp was excellent and increased steadily increased with lower to higher concentrations (50 - 250µl/ml), which were shown in Table 1. The highest reducing powers were found in250 µl. The difference scavenging effect among solvent extraction was due to that free radical scavenging activity is species-dependent. Besides, most the scavenging activities were probably due to light sensitivity of the DPPH radical although with variations of extracts (**Masalu *et al.*, 2012**).

The results of the antimicrobial activity ranged from 12mm to 26mm zone of inhibition against all five bacterial pathogens. Minimum zone of inhibition 12mm was observed in 50 µl concentration against ***Streptococcus pyogenes*** and maximum zone of inhibition 26mm was observed in 200µl concentration against ***E. coli*** shown in the table no 2.

In the 50µl concentration ***Streptococcus pyogenes*** showed lowest zone of inhibition(12mm) and highest zone of inhibition(22mm) was observed against ***E. coli***.

The 100µl concentration of extract resulting the lowest zone of inhibition (13mm) was observed against ***Streptococcus pyogenes*** and ***Pseudomonas aeruginosa. E. coli*** showed highest zone of inhibition (23mm).

In the 150 µl concentration of extract the lowest zone of inhibition(14mm) was observed against ***Streptococcus pyogenes. E.coli*** presented highest zone of inhibition(23mm).

In the 200 µl concentration the minimum zone of inhibition(15mm) was observed against ***Streptococcus pyogenes*** and maximum zone of inhibition(26mm) was observed against ***E. coli*** shown in figure no 3 and 4.

Compare to studies of **chalalai et al (2015**) the extracts showed higher inhibitory effect against gram positive bacteria than gram negative bacteria. In our study gram negative bacteria showed higher inhibitory effect than gram positive bacteria against methanol extract of coffee pulp.

IV. Conclusion:

The study has been carried out to develop natural and cost-effective material from agro-industrial residue. The results of this study confirmed that the coffee pulp had the potential of antimicrobial and antioxidant activity, but require further studies to optimize the extraction methods to recover larger amounts of biologically active compounds.

These results are experimental evidence of the potential of using coffee pulp as a natural source of antioxidants which can be extracted through fermentative and enzymatic processes (***Chalalai JAISAN et al 2015)*.** Further studies are in progress individual phenolic regarding the characterization of underlying compounds and to elucidate the mechanism bioactive properties and existence of possible synergism, if any, among these compounds.

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