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OPTIMIZATION AND COMPARISON OF BANANA PEEL EXTRACT WITH TINOSPORA CORDIFOLIA FOR ANTIOXIDANT STUDIES

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ABSTRACT

In this study, the extracts from Musa Paradisiaca L. (Musaceae) and Tinospora Cordifolia (Thunb).Miers.(Menispermaceae) were obtained and compared. The influence of various factors such as type of Drying techniques employed(Natural Drying, Lyophilisation), type of the Solvent used(ethanol, acetone, xylene, ethyl acetate and hexane),Concentration range, Dosage, Extraction method(Liquid-Liquid Extraction, Soxhlet Extraction, and Aqueous Two Phase Extraction) were investigated and optimal experimental conditions were ascertained, qualitative, quantitative analysis of the extract was carried out and the purity was being estimated by carrying out Gas chromatography Method.

KEYWORDS: Musa Paradisiaca, Tinospora Cordifolia, Lyophilisation, Soxhlet Extraction.

I. INTRODUCTION

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Many treatment options for cancer exist, with the primary ones being surgery, chemotherapy, radiation therapy, hormonal therapy, targeted therapy and palliative care.[1] Most of these treatments are painful and may cause side-effects in the human body and further they are very expensive.

Natural antioxidant particularly found in fruits and vegetables have gained a lot of interest among consumer and the scientific community because epidemiological studies have indicate that frequent consumption of natural antioxidant is associate with the lower risk of

cardiovascular and cancer. Natural antioxidants are perceived safe, less toxic and beneficial for human health.

Many agricultural wastes can also be used for the treatment of cancer such as peels of banana, citrus fruits etc. Among them the peels of Banana is said to have certain antioxidant.[2] and anticancer/ Immunomodulatory properties.[3] Further it is also used in the treatment of acne, warts, teeth whitening, water filtration, scraps, scratches, rashes and itches, etc and they are enriched with as vitamins (A, B and E), β–carotene,selenium, minerals like potassium, phosphorus, magnesium, calciumand phenolic compounds like catechin, epicatechin, lignin, tannin, gallocatechin and anthocyanins like peonidin,malvidine, This peel is biodegradable and it will produce environmental problem due to it nitrogen and phosphorus quantity. Therefore, extracting the banana peel will be the best solution in order to protect human being, gaining some profit and creating waste to wealth. According to National Cancer Standard Institute banana peel extract is described as non toxic to normal human cells, so it can be used as a natural source of antioxidants.[4]

Guduchi (T. cordifolia) is widely used in veterinary folk medicine/ ayurvedic system of medicine for its general tonic, antiperiodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-allergic and anti-diabetic properties.[5] The plant is used in ayurvedic, "Rasayanas" to improve the immune system and the body resistance against infections. The root of this plant is known for its antistress, anti-leprotic and anti-malarial activities.

In the present study the main motto/objective is to establish the optimal conditions(drying techniques, types of solvents, type of extraction and concentration) for obtaining the banana peel (Musa paradisiaca) extract for antioxidant,anticancer properties and comparing the results with that of Tinospora cordifolia stem for the same. This will proximate study of effect of banana peels and tinospora cordifolia, the optimization of parameters, effects and changes, extent and efficiency.

2. MATERIALS AND METHODS

Chemicals and Reagents

Ethanol, Xylene, Hexane, Distilled water, Ethyl acetate, Acetone, 20% Na_2CO_3, Folin-Ciocalteau reagent,20 mM Phosphate buffer, 1% w/v Potassium Ferricyanide, 10% w/v Trichoroacetic acid, 0.1% w/v Ferric chloride.

Samples Collection

Musa paradisiaca peels and Tinospora cordifolia were collected from the local market of Belagavi. The plants were authenticated from Regional Medical Research Centre (RCMR), Belagavi.

Taxonomic classification of Musa paradisiaca

Kingdom: Plantae

Division: Tracheophyta, vascular plants, tracheophytes

Class: Magnoliopsida

Order: Zingiberales

Family: Musaceae-banana

Genus: Musa L -banana

Species: Musa X paradisiaca L. (pro sp.) –banana, plantain, French plantain

Taxonomic classification of Tinospora Cordifolia

Kingdom: Plantae

Class: Magnoliopsida

Order: Ranunculaceae

Family: Menispermaceae

Species: Tinospora cordifolia

EXPERIMENTAL METHODOLOGY

1) Pre-treatment of M. paradisiaca peels & T. cordifolia stem

The peels obtained were carefully separated from the pulp,rinsed with distilled water and kept to dry at room temperature for a few minutes and then put into their respective drying methods.Tinospora cordifolia stem was further cut into smaller fragments to achieve faster drying.

2) Drying of samples

Three drying techniques were incorporated viz. drying at room temperature (25°C -30°C), drying in hot air oven (65°C -70°C) and lyophilisation (-50°C).[6] Samples were kept in such conditions till a powder could be obtained and then they were powdered using the pestle and mortar and stored at low temperatures in a refrigerator.

3) Preparation of Crude Extracts

i) Preparation of sample extracts in solvents

For estimation of polyphenol, flavonoids and antioxidant activity, samples (M. paradisiaca and T. cordifolia; dried by lyophilisation, hot air oven and sun) were extracted with ethanol, hexane, xylene, acetone and ethyl acetate medium. 1.0 g of sample was suspended with 100 ml solvent, centrifuged for 3000 rpm for 10 minutes and filtered. All analysis was carried out in freshly collected extracts.

ii) Preparation of Aqueous Extract

Freshly collected Musa paradisiaca peels and Tinospora cordifolia were dried by hot air oven & sun dried techniques, coarse powder was prepared by maceration in water for one week. Extract was then centrifuged for 3000 rpm for 10 minutes and filtered. This aqueous extract was given for analysis of antioxidants at BSCR, Belagavi.

4) Analysis of Total Polyphenol Content

Samples were analyzed for total polyphenol content according to the Folin-Ciocalteu method.[7] To 0.5 ml aliquot of the extract solution, 0.2 ml of Folin-Ciocalteau reagent, and a saturated solution of Na_2CO_3 (0.5 ml) was added. This was increased to 10 ml with distilled water and incubated at 27°C for 30 min. Optical density was measured at 765 nm using a spectrophotometer. The concentration was calculated using tannic acid as a standard and the results were expressed as tannic acid equivalents/100 g of sample.

5) Determination of antioxidant activity by using In-vitro methods

i) Folin-Ciocalteu reagent (FCR) assay: To estimate the Phenolics content

Procedure

Aqueous extract of M. paradisiaca and T. cordifolia in different concentration range from 100 µl to 500 µl were added to each test tube containing of 900 µl to 500 µl distilled water respectively; and 500 µl of Folin-Ciocalteu reagent solution. 500 µl of 100 mg/ml sodium carbonate was added after 5min. These tubes were kept aside for 2 hrs. Absorbance was measured at 765 nm. The concentrations of phenolic compounds in M. paradisiaca and T. cordifolia aqueous extracts were expressed as gallic acid equivalents (GAEs). All assays were conducted in triplicate and its mean was calculated.

ii) Ferric ion reducing antioxidant power assay (FRAP)

Ferric ions reducing power was measured according to the method of Oyaizu.[8] with a slightest modification.

Procedure

Aqueous extract of M. paradisiaca and T. cordifolia in different concentrations ranging from 100 µl to 500 µl were mixed with 2.5 ml of 20 mM phosphate buffer and 2.5 ml 1%, w/v potassium ferricyanide, and then the mixture was incubated at 50 °C for 30 min. Afterwards, 2.5 ml of 10%, w/v trichloroacetic acid and 0.5 ml 0.1%, w/v ferric chloride were added to the mixture, which was kept aside for 10 min. Finally, the absorbance was measured at 700 nm. Ascorbic acid was used as positive reference standard. All assays were run in triplicate way and averaged.

6) Gas Chromatography-Mass Spectrometry

GC-MS analysis.[9] was carried out on a GC 7890 (Agilent) comprising automatic liquid sampler and gas chromatograph interfaced to mass spectrophotometer (GC-MS) at BSRC. Helium was used as a carrier gas and the injector temperature was kept at 350°C. The oven temperature was programmed from 100°C held for 5 mins to 375°C at 20°C /min. The name, molecular weight and structure of the component were ascertained.

3. RESULTS AND DISCUSSIONS

Based on the partition coefficient the solvents was chosen and FCR assay for the samples of Musa Paradisiaca and Tinospora Cordifolia which were Lyophilised, Sundried and kept in Hot Air Oven were subjected to Soxlet Extraction using acetone, hexane, ethyl acetate solvents as to determine the total phenolic content and thereby the Optical Density values were being obtained at 765nm.

1) FCR for total phenolic content using Acetone as Solvent:

Samples were subjected to Soxlet Extraction process using acetone as solvent and the Optical Density was obtained at 765nm. It is seen from Table-1 that the Lyophilised green peel of banana has the highest optical density of 0.305 when compared to the lyophilized yellow peel of banana and lyophilized Tinospora Cordifolia Samples. It is also seen that the Hot air dried and Sundried sample of Tinospora Cordifolia has the highest O.D of 0.332 and 0.401 when compared to lyophilized yellow and green peel of banana.

2) Ethyl acetate as Solvent

Samples were subjected to Soxlet Extraction process using Ethyl Acetate as solvent and the Optical Density was obtained at 765nm.It is seen from Table-II that the Lyophilised and Sundried samples Tinospora Cordifolia has the highest optical density of 0.315 and 0.398 when compared to the lyophilized yellow and green peels of banana Samples. It is also seen that the Hot air dried sample of yellow peel of banana has the highest O.D of 0.464 when compared to hot air dried sample of green peel of banana and Tinospora Cordifolia.

3) Antioxidant Testing

i) FCR Assay

As phenolics contribute to the reducing capacity so much focus is on the phenolics concentration determined by FCR method. FCR assay was used to quantify the reducing capacity of antioxidant. It was suggested to define as FCR method better rather than as total phenolics content of antioxidant as shown in Figure 1.Different samples (Sundried and HAO of Musa Paradisiaca (green and yellow peels) and Tinospora Cordifolia in the volume range of (100 µl, 200 µl, 300 µl,400 µl,500µl) were taken and was subjected to FCR assay for antioxidant testing and the Optical Density was obtained. From the Table III it is clear that the Sundried and HAO green peel sample of banana has the highest O.D of than Sundried yellow peel of banana and Tinospora Cordifolia.

ii) FRAP Assay

FRAP measures the reducing potency of extract and standard antioxidant. Higher absorbance indicates higher reducing potency. Different samples(Sundried and HAO of Musa Paradisiaca (green and yellow peels) and Tinospora Cordifolia in the volume range of (100 µl,200 µl,300 µl,400 µl,500µl) were taken and was subjected to FRAP assay for antioxidant testing and the Optical Density was obtained. From the Table IV it is clear that the Sundried green peel sample of banana and has the highest O.D of than Sundried yellow peel of banana and Tinospora Cordifolia.

Table I: Total phenolic content by FCR method, acetone as solvent

Sl. No.	Sample	O.D at 765nm
1	Lyophilized Tinospora Cordifolia	0.315
2	Hot air dry Tinospora Cordifolia	0.462
3	Sundry Tinospora Cordifolia	0.398
4	Lyophilized Green Peel of Banana	0.112
5	Hot air dry green Peel of Banana	0.282

6	Sundry Green Peel of Banana	0.197
7	Lyophilized yellow Peel of Banana	0.204
8	Hot air dry yellow Peel of Banana	0.464
9	Sundry yellow Peel of Banana	0.245

Table II: Total phenolic content by FCR method, ethyl acetate as Solvent

Sl. No.	Sample	O.D at 765nm
1	Lyophilized Tinospora Cordifolia	0.229
2	Hot air dry Tinospora Cordifolia	0.332
3	Sundry Tinospora Cordifolia	0.401
4	Lyophilized Green Peel of Banana	0.305
5	Hot air dry green Peel of Banana	0.252
6	Sundry Green Peel of Banana	0.289
7	Lyophilized yellow Peel of Banana	0.224
8	Hot air dry yellow Peel of Banana	0.172
9	Sundry yellow Peel of Banana	0.180

Table III: FCR assay for antioxidant testing using aqueous extract of sample

Volume (µl)	Absorbance at 765 nm						
	Sundry green peel of Banana	HAO Green peel of Banana	Sundry yellow peel of Banana	HAO Yellow peel of Banana	Sundry Tinospora Cordifolia	HAO Tinospora Cordifolia	Standard
100	0.150	0.111	0.119	0.020	0.060	0.105	0.276
200	0.303	0.193	0.233	0.040	0.131`	0.215	0.556
300	0.452	0.296	0.360	0.082	0.189	0.320	0.827
400	0.504	0.415	0.471	0.124	0.252	0.432	1.024
500	0.660	0.420	0.590	0.144	0.306	0.543	1.305

Table IV: FRAP assay for Antioxidant Testing Using Aqueous Extract of Sample

Volume (µl)	Absorbance at 765 nm						
	Sundry green peel of Banana	HAO Green peel of Banana	Sundry yellow peel of Banana	HAO Yellow peel of Banana	Sundry Tinospora Cordifolia	HAO Tinospora Cordifolia	Standard
100	0.361	0.141	0.283	0.006	0.060	0.187	0.449667
200	0.377	0.222	0.325	0.013	0.110	0.236	0.473333
300	0.436	0.343	0.391	0.029	0.145	0.374	0.493
400	0.544	0.398	0.411	0.062	0.177	0.434	0.536
500	0.595	0.509	0.463	0.126	0.208	0.542	0.662

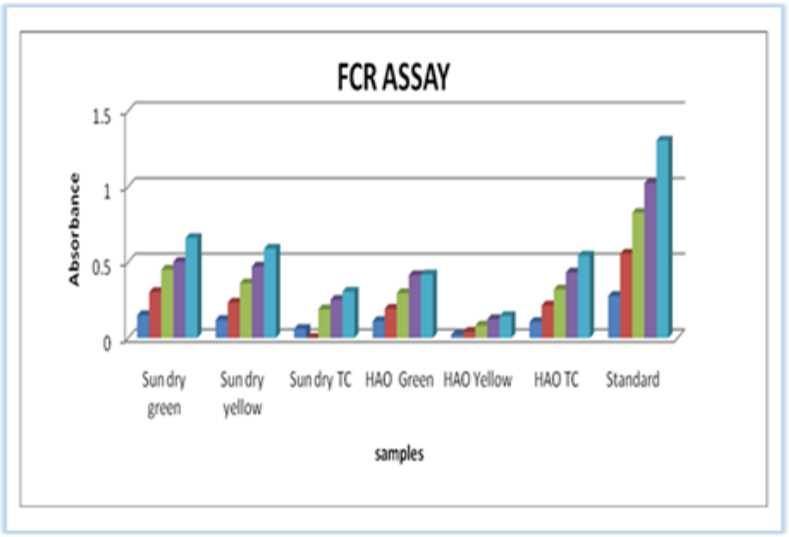

Figure 1: Graphical representation of FCR assay for antioxidant testing, aqueous extract of sample

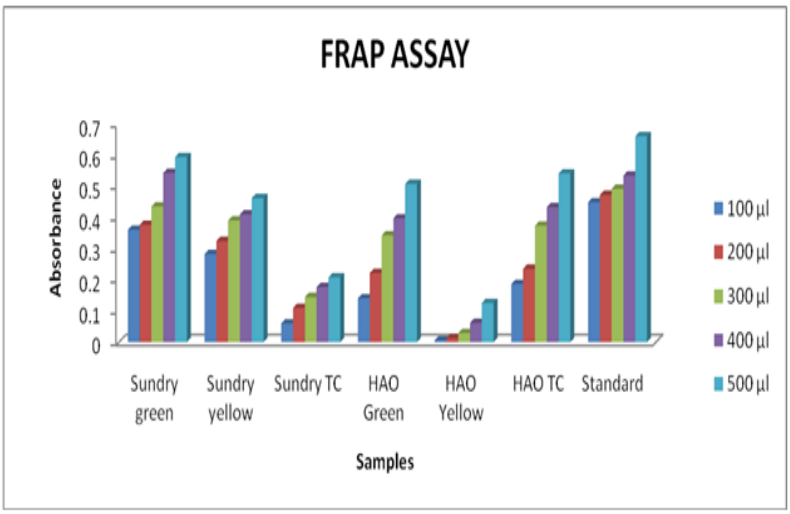

Figure 2: FRAP assay for Antioxidant Testing, Aqueous Extract of Sample

4)Gas Chromatography- Mass Spectroscopy

Table V: Retention Time of Bioactive Compounds obtained from GC – MS

Sl. No.	Retention Time	Area (%)	Name of the Compound
1	5.0	10.33	Estragole
2	16	6.01	Gallocatechin
3	20	4.39	Lycopene
4	25	14.96	Berberene
5	28	19.01	Beta-tocopherol

4. COST ESTIMATION

The cost of banana is Rs 500, Chemicals/Reagents is Rs 1000/- and the cost of analysis of samples is Rs 2400/-.

5. CONCLUSION

From the results obtained, it could be concluded that the extract from the peels of the Musa paradisiaca which were sundried showed the highest phenolic content and whereas the stem of Tinospora cordifolia which were dried in hot air oven has the highest phenolic content. The results of the FCR and FRAP assay for antioxidant testing showed that the phenolic content in the green peels of Musa paradisiaca had the highest content which corresponded to the amount of antioxidants present in it in comparison with that of Tinospora cordifolia. A study was also carried out on the immuno-modulatory properties of Musa paradisiaca (study on lectin) and Tinospora cordifolia.Also it was found that Tinospora cordifolia was found to be more efficient and cost effective in comparison with the Musa paradisiaca.

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7. REFERENCES

1. Akulapalli Sudhakar. History of Cancer, Ancient & Modern Treatment Methods. Journal of Cancer Science & Therapy, 2009; 1(2): 1-4, doi: 10.4172/1948-5956.100000e2
2. Bashir Ado Ahmad, Khamsah Suryati Mohd,Muhammad Abdurrazak,U.S.Mahadeva Rao,Thant Zin. Phytochemical Screening, Antioxidant activity of pure syringing in comparison to various solvent extracts of Musa Paradisiaca(Banana) & total phenolic contents. International Journal of Pharmacy & Pharmaceutical Sciences, 2015; 7(5): 242-247.

3. Manmohan Singhal, Purnima Ratra. Investigation of Immunomodulatory Potential of methanolic & hexane extract of Musa Acuminate peel extracts. Global Journal of Pharmacology, 2013; 7(1): 69-74, DOI: 10.5829/idosi.gjp.2013.7.1.71145
4. Hyc Lee, Hye-Jung Yeom, Mi-Sun Ha, Dong Ho Bae. Development of banana peel jelly & its antioxidant & textural properties. Journal of Food Science & Technology, 2010; 19(1): 449-455. doi:10.4172/2155-9600.1000370.
5. S.S.Singh,S.C.Pamdey,S.Srivastava,V.S.Gupta,B.Patro,A.C.Ghosh. Chemistry & medicinal properties of Tinospora Cordifolia(Guduchi).Indian Journal of Pharmacology, 2003; 35: 83-91.
6. Norshahida Mohamad Shofian, Azizah Abdul Hamid, Azizah Osman, Nazamid Saan,Farooq Anwar, Mohd Sabri Pak Dek,Muhammad Redzuan Hairuddin. Effect of Freeze-drying on antioxidant compounds & antioxidant activity of selected tropical fruits. International Journal of Molecular Sciences, 2011; 12(7): 4678-4692, doi: 10.3390/ijms12074678.
7. Andressa Blainski, Gisely Cristiny Lopes, Jaao Carlos Palazzo Dimello. Application & Analysis of Folin Ciocalteu method for determination of total phenolic content from Limonium Brasiliensel.International Journal of Molecules, 2010; 18: 6852-6865, doi :10.3390/molecules18066852.
8. Aparadh V.T,Naik V.V, Karadge B.A. Antioxidative Properties [TPC, DPPH, FRAP, Metal Chelating Ability, Reducing Power & TAC) within some cleome species. Journal of Annali Di Botanica, 2012; 2: 49-56. doi: 10.4462/annbotrm-9958.
9. S.Gopalkrisnan,E.Vadive. GC-MS analysis of some bioactive constituents of Mussaenda Frondosa Linn,International Journal of Pharma & Biosciences, 2011; 2(1): 313-329.