INVESTIGATION OF ADSORPTION EFFECTIVENESS OF KOKUM *(GARCINIA INDICA)* RIND RESIDUE ON SYNTHETIC DYES AND DETERMINATION OF BEST EXTRACTION PROCEDURE FOR KOKUM PHENOLS

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ABSTRACT

The aim of the study is to investigate the potential use of kokum fruit for production of natural dye and scrutinizing the adsorption effectiveness of the residual with respect to time, dye concentration, adsorbent dosage and pH. Extraction of dried fruit rind of kokum was carried out by using various enzymatic methods and other modern techniques such as Microwave assisted extraction and Ultrasonication. Total phenolic content in fruit extract of Garcinia Indica was found to be 0.599GAE (Enzyme assisted) > 0.582GAE (Ultrasonication) > 0.461GAE (Microwave assisted) respectively in terms of Gallic acid equivalent (GAE). Adsorption studies were further made on the residual fruit rind under various operating parameters. Experimental data indicated that the adsorption capacity was dependent on operating variables such as contact time, p^H, adsorbent dosage and dye concentration. The contact time required to attain equilibrium was around 120min.The adsorption data were correlated with the Freundlich and Langmuir adsorption isothermal models and were found satisfactory. All extracts were concentrated via Vaccum Evaporation and made free of solvents which have been used for extraction and the final concentrate obtained was used to dye cloth. Positive antimicrobial activity of kokum extract was documented for three strains viz. E.coli, Bacillus subtilis and Staphylococcus aureus.

Keywords: Adsorption, Dyes, Extraction, Garcinia, Total Phenolics etc

I INTRODUCTION

Garcinia indica or commonly known as Kokum, one of several species of Garcinia is distributed mainly in peninsular India. Kokum is grown in tropical rain forest of Western ghats in konkan, Goa, South Karnataka and Kerela.[7] The extract of the fruit has both antifungal and antibacterial properties and therefore, has a potential for use as bio preservative in food applications. Many therapeutic effects of the fruit have been described in traditional medicine based on Ayurveda.[2] Kokum Butter is an excellent emollient used by the cosmetic industry for preparations of lotions, creams, lip-balms and soaps. It is extracted from the Kokum seed and is supposed to reduce degeneration of the skin cells and restore elasticity.[1]

Garcinia indica extracts, especially from its rind, are rich in phenols like polyisoprenylated benzophenone derivatives such as Garcinol and its colorless isomer, Isogarcinol. Garcinol shows strong antioxidant activity since it contains both phenolic hydroxyl groups as well as a β-diketone moiety, and in this respect it resembles with the well known antioxidant of plant origin, viz. Curcumin The rind also contains hydroxycitric acid (HCA), hydroxycitric acid lactone, citric acid and oxalic acid. HCA from the rind of the fruit is used as a hypocholesterolaemic agent.and is also a potential anti-obesity agent. It suppresses fatty acid synthesis, lipogenesis, and food intake, and thus induces weight loss. Anthocyanin the coloring pigment is used in food industry as a coloring agent and has anti-oxidant activity. The structures of these compounds are shown in Figure 1. [12]

The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter- current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydrofluorocarbon solvents). The variations in different extraction methods that will affect quantity and secondary metabolite composition of an extract depend upon type of extraction, time of extraction, temperature, nature of solvent, solvent concentration, polarity [5,6]

Surveys on the process of extracting acid from dried rinds of *Garcinia oblongifolia* Champ. ex Benth (*G.oblongifolia*) using microwave in terms of time, machine power and solid/liquid rate, have resulted in such findings as: the best time allocation for extracting is 25 minutes; machine power works best at level 2 (microwave power is 400 W); suitable rate of solid/liquid is 0.071 (approximately 150 mL solvent per 10 g of sample). The total amounts acid and (-)-hydroxycitric acid extracted from 100 g dried rinds of *G. oblongifolia* are 18.592 g and 10.137g respectively. This is the first finding on extraction of (-)-HCA from dried rinds of *G. oblongifolia* using microwave**.** [12] The raw, dry fruit powder was extracted with 99.9% of methanol. Phytochemical test shows that extract contains higher level of total phenol and flavonoids the methanolic extract of *Garcinia indica* fruit could be considered for prevention and treatment of human diseases and its complications as potent antioxidant .[1]

Use of kokum extract in dyeing of clothes using various methods was carried out. It has been seen that kokum has a strong antimicrobial activity against a wide range of Gram positive and Gram negative bacteria. It also shows veridical and fungicidal activity when in contact for a longer period. As the kokum peel contains many important phyto chemicals, the emphasis of the present study has given to the use of it as a remedy for inhibition of various pathogenic and non-pathogenic bacteria. Antimicrobial activity of various extracts on pathogenic strains Bacillus *subtilis, Escherichia coli, Staphylococcus aureu*s was examined. The high antioxidant activity of kokum adds one more positive attribute to its known medicinal properties and hence its use in cooking, home-remedies and as a soft drink may be promoted. [1] The antioxidant potential of *Garcinia indica* could be responsible for its potential benefits in ameliorating number of diseases. This is actually known for its anti-inflammatory actions, but is significantly

antibacterial in its characteristics against gram positive bacteria. That is why the extracts of kokum are often used to cure dermatitis or other mild skin infections, when applied topically. [11]

Dyes have long been used in dyeing, paper and pulp, textiles, plastics, leather, cosmetics and food industries. Color stuff discharged from these industries poses certain hazards and environmental problems. These colored compounds are not only aesthetically displeasing but also inhibiting sunlight penetration into the stream and affecting aquatic ecosystem. A mordant is a substance used to set dyes on fabrics or tissue sections by forming a coordination complex with the dye which then attaches to the fabric or tissue. It may be used for dyeing fabrics, or for intensifying stains in cell or tissue preparations.[8] Dye results can also rely on the mordant chosen as the introduction of the mordant into the dye will have a marked effect on the final color. Each dye can have different reactions to each mordant. [2] Dyes usually have complex aromatic molecular structures which make them more stable and difficult to biodegrade. Furthermore, many dyes are toxic to some microorganisms and may cause direct destruction or inhibition of their catalytic capabilities. [2] Due to their chemical structure, dyes possess a high potential to resist fading on exposure to light and water. The main sources of wastewater generated by the textile industry originate from the washing and bleaching of natural fibers and from the dyeing and finishing steps. Given the great variety of fibers, dyes and process aids, these processes generate wastewater of great chemical complexity and diversity, which are not adequately treated in conventional wastewater treatment plant. So there is a need of finding dye of natural origin which will cause less cause to environment. [2]

There are various conventional methods of removing dyes including coagulation and flocculation, oxidation or ozonation and membrane separation. However, these methods are not widely used due to their high cost and economic disadvantage. Chemical and electrochemical oxidations, coagulation are generally not feasible on large scale industries. In contrast, an adsorption technique is by far the most versatile and widely used. The most common adsorbent materials are: alumina silica, metal hydroxides and activated carbon. There is a scope of finding an alternate adsorbent which can be widely used as an adsorbent due to its high adsorption capacity, high surface area, micro porous structure, and high degree of surface respectively.[3]

Objectives of the present study are 1) To extract phenolics from Kokum fresh fruit, peel, and dried whole fruit using three methods of extraction i.e. microwave, enzyme and ultrasonication with varying concentration of methanol solvents viz.0,20,40,60,80,100 in% . 2) To compare and determine the yield of extraction. 3) To investigate the adsorption property of kokum rind residue on synthetic dyes by varying parameters like time, pH, concentration of adsorbate and adsorbent. 4) Explore antioxidant properties of *Garcinia indica* alcoholic fruit extract**.**

2 Materials and Methods:

2.1 Collection of Fruits:

The fresh fruits of *Garcinia indica* were collected from kokan region of Maharashtra. Since kokum is a seasonal fruit, the rinds and fruit of kokum fruits are usually preserved by salting and sun drying. These preserved rinds and fruit were collected and oven dried for 48 hour at 45˚C. After the peels were dried thoroughly, they were ground to coarse powder with the help of an electric grinder and were stored for further use. The coarse powder was then used for three different extraction processes viz. ultrasonication, microwave extraction and enzyme assisted extraction. Solvent used for extraction of phenol was Methanol at various concentrations viz. 0%, 20%, 40%, 60%, 80%, 100%.

3. Extraction methods of Phenols:

3.1. Ultrasonication:

In this extraction method 5 gm of dried peel powder & fresh fruit was taken separately in different conc. of solvent methanol (50ml) Incubate sample at varying methanol concentration viz.0, 20, 40, 60, 80,100 (%) for 1 day at room temperature. Ultrasonication was performed on the prepared sample for 30 min. The sample was filtered. The filtrate and residue was stored at 4˚C for further analysis.

3.1.2. Microwave Extraction :

5 gm of dried peel powder & fresh fruit was taken separately in different conc. of solvent methanol (50ml). The sample was incubated at varying methanol concentration viz. 0, 20, 40, 60, 80,100 (%) for 1 day at room temperature. The sample was then microwaved at 700W for 90 sec. Further filtration was done and filtrate was analysed it for total phenolic content at 760 nm from standard Gallic acid equivalent test.

3.1.3. Enzyme Extraction method:

5 gm of dried peel powder & fresh fruit was taken separately in (50 ml) PBS buffer (pH- 4.5) Heat shock treatment for 10 min & cooled it in an ice bath. Addition of 2% cellulase enzyme and 50ml of propanol was done in a beaker. Stirring was given for 24 hrs. using magnetic stirrer. Deactivation of the enzyme was carried out using heat treatment and the sample was filtered and stored for further analysis.

4. Screening of Phenols:

The total phenolic content of *Garcinia indica* was estimated according to the method using Folin- Ciocalteu reagent. The aliquots of the extract was taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially to the test tube. Soon after over testing the reaction mixture, the tubes were placed in the dark for 40 min. and the absorbance was recorded at 725 nm against the reagent blank. Using Gallic acid monohydrate, a standard curve was plotted.

4. Adsorption Studies:

The residue obtained after extraction was washed and oven dry .The dyes used were Commassieve Brilliant Blue, Congo Red, Malachite Green, Nigrocin, Saffranin. 1 gram of the residue was treated with dyes and estimated for following parameters:

- varying time viz.0, 30, 60, 90,120,150 minute
- pH (3, 6, 9)
- at varying concentration (mg/ml) of dye
- at different concentration of adsorbent

Fig 3. Different concentration of Malachite Green dye before and after Adsorption

4.1. Effect of Contact time:

150 ml of dye solution with dye concentration (50mg/L) was prepared in a conical flask with adsorbent concentration (0.5g/10ml) and kept inside the shaker. Dye concentration was then estimated spectrophotometrically at the wavelength corresponding to maximum absorbance, λmax, using a UV Visible Spectrophotometer. The samples were then withdrawn from the incubator shaker at predetermined time intervals and the dye solution was then separated from the adsorbent by the help of a micropipette. The dye concentration was measured after 5, 10, 20, 30,60, 90,120 min until equilibrium reaches. A graph was plotted with q_e vs time. The q_e is expressed as

$$q_e = \frac{C_e - C_o}{X} \quad \text{---------- (1)}$$

Where, q_e = Amount of dye adsorbed per unit mass of adsorbent (mg/g).

C_0= Initial dye concentration (mg/L).

Ce= Final dye concentration (mg/L).

X = Dose of adsorbent (g/L).

4.2.Effect of p^H and Concentration of Dye:

150ml of dye solution was prepared in a conical flask with dye conc. 50mg/L and adsorbent conc. (1g/10ml) and initial pH of the conical flask was measured. The pH of the dye solutions was adjusted with dilute HCl (0.05N) or NaOH (0.05N) solution by using a pH meter. 150 ml of dye solution was prepared taking the above dyes and the pH of solution is changed from 3 to 9.The flasks were put inside the incubator shaker (120rpm fixed throughout the study) maintained at 27˚C and the final concentration of dye was measured using UV spectrophotometer and the calibration plot of the dye after 2 hours. A graph was plotted with q_e vs initial p^H.

4.3 Effect of Adsorbent dose:

150ml of dye solution was prepared in different conical flasks with dye conc. (50mg/L) and adsorbent concentration 0.25, 0.5, 1, 1.25, 1.5, grams/10ml. The final dye concentration readings were taken after putting flasks inside the shaker for 2 hours. A plot of q_e vs adsorbent dose is taken.

5. Extraction and testing of natural dye:

Dyeing is the process of adding color to textile products like fibers, yarns, and fabrics.The filtrate obtained after extraction was concentrated using Rotavap Evaporator at 80˚C for 45 minutes. It was tested as a dye along with mordents like alum, vinegar for dyeing of clothes like cotton, teflon, wool by pre, post and simultaneous mordanting method. In pre mordanting method 10ml of mordant (vinegar, alum) was treated on Teflon, cotton and woollen cloths respectively for 1 hour at 80˚C. After treatment with mordant the cloths were sun dried and then 10ml of concentrated dye was added to the cloths on heating water bath. After 1 hr treatment the cloth was washed with salted water and sun dried and checked for its colour. In post mordanting method 10ml of dye was treated on Teflon, cotton and woollen cloths respectively for 1 hour at 80˚C on a boiling water bath. After that it was dried and then dipped into 10ml of mordents (vinegar, alum) for 1 hour at 80˚C. Cloths were then washed with salted water and sun dried and checked for its dyeing colour. In simultaneous mordanting method both the dye and mordant were added at the same time to the cloths and kept for 1 hr at 80˚C. After treatment the cloth was washed with salted water and sundried and checked for its dyeing colour.

6. Antimicrobial activity:

Most of the compounds in *Garcinia indica* peel have various biological activities with potential health benefits, such as antibacterial, antiviral, wound healing, antioxidant and hence its antimicrobial activity against various pathogenic bacterial strains was determined. Strains were obtained from the laboratory and were sub cultured on the agar medium so as to use the strains for further experimentation. Nutrient medium agar is mainly used for this purpose. The agar was prepared in a flask and its mouth was covered with a cotton plug in order to avoid any contamination.

Six petri plates, micropipette tips etc. were wrapped with a filter paper and were sterilized in an autoclave. There were two methods viz. well diffusion method and disc diffusion method with which antimicrobial activity of *Garcinia indica* peel was determined.[11]

6.1. Well Diffusion Method:

In this method agar is mixed with the microbial strain and poured in the petri plate under sterilized conditions. After the agar gets solidified two holes are punched in the agar. The fluid extract is then added in one well and distilled water is added in the other well as a control. The process is repeated for each strain individually; plates are marked with the names of respective organisms and kept in an incubator overnight. Plates are then checked for any antimicrobial activity which is determined by measuring the zone of inhibition around the well. Due to the disadvantage of no uniform spreading the antimicrobial activity cannot be determined exactly, hence disc diffusion method was mainly used.

6.2. Disc Diffusion Method:

This method is mostly used as organisms can be spread uniformly on the agar; it has a drawback of getting contaminated easily which can be reduced by working in highly sterilized conditions. Sterile Nutrient medium agar was poured in the plates. The bacterial test organisms like Bacillus *subtilis, Staphylococcus aureus, E.coli were* spread over Nutrient medium agar plates using separate sterile glass spreader from the stock solution prepared in saline water. Holes were punched on the plates and labeled. Ampicillin was taken as control and different concentrations of samples viz.20, 40, 60, and 80,100 were poured in the wells using micropipette. The plates were then incubated at 30°C for 24 h. in an incubator. The diameter of the minimum zone of inhibition was measured in mm. Each test was performed in replicates. The antimicrobial assay was performed individually for the extracts from ultrasonication for 20, 40, 60, 80, 100 % extract.

7. Results and Discussion:

7.1. Comparative study of effect of source, extraction method and concentration of solvent on extraction of phenols:

The comparative study was carried out on the extraction of phenols from *Garcinia indica peel*, fresh fruit, and dried whole fruit by using three methods of extraction which showed presence of medicinally active compounds in the extract carried out at different concentrations of methanol viz.0, 20, 40, 60, and 80,100%. Detection of the phenolic compounds was done using phenols screening assay using Folins-Ciocalteau reagent .The calculation for yield obtained from different sources, extraction method and concentration of methanol was done by plotting Gallic acid standard graph and comparing that GAE/mg of sample was obtained. Presence of phenols were characterized by change in color of from pale yellow to greenish blue. Result were obtained from spectrophotometric analysis at 720 nm.

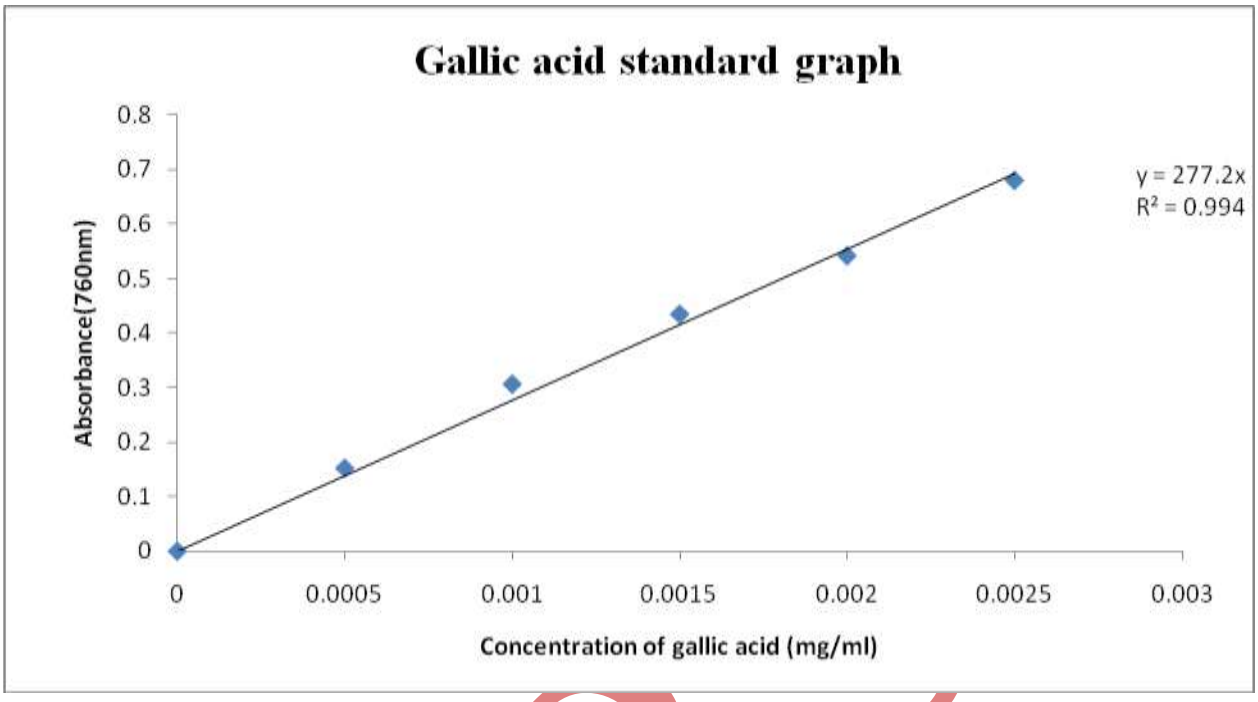

Fig. 7.1.1: Standard Graph for Total Phenolic Content in Methanol solvent

The yield obtained from total phenolic content was calculated by plotting Gallic acid standard graph. The slope obtained was used to calculate Gallic acid equivalent i.e. GAE/mg of sample**.** The formula is as follows:

$$\text{Dilution factor} = \frac{\text{Absorbance(nm)}}{\text{Slope of Graph}} \text{ X Yeild(GAE/mg)} \quad \text{---------- (2)}$$

Table 7.1.1: Phenols Analysis of Extracts obtained using ultrasonic extraction

Conc. of methanol (%)	Fresh fruit absorbance	GAE/mg of sample	Dry rind absorbance	GAE/mg of sample	Whole dry fruit	GAE/mg of sample
100	0.383	**0.703**	0.323	0.582	0.280	0.505
80	0.224	0.403	0.180	0.324	0.156	0.281
60	0.133	0.239	0.08	0.194	0.101	0.182
40	0.128	0.230	0.106	0.191	0.098	0.160
20	0.124	0.223	0.104	0.187	0.095	0.170

By ultrasonic extraction method, aqueous extract gave positive results for the presence of phenols at different concentrations of methanolic kokum extract. Of these 100 % methanolic extract showed maximum extraction of phenols followed by 80, 60, 40, and 20%. Fresh fruit showed highest concentration of phenols i.e. 0.703 GAE/mg of sample as compared to dry rind and whole dry fruit.

Table 7.1.2: Phenolic Analysis of Extracts obtained using microwave extraction

Conc. of methanol (%)	**Fresh fruit absorbance**	**GAE/mg of sample**	**Dry rind absorbance**	**GAE/mg of sample**	**Whole dry fruit**	**GAE/mg of sample**
100	0.272	**0.490**	0.256	0.416	0.226	0.407
80	0.123	0.222	0.116	0.209	0102	0.183
60	0.109	0.196	0.103	0.185	0.091	0.164
40	0.107	0.193	0.101	0.182	0.086	0.160
20	0.104	0.187	0.098	0.160	0.086	0.155

By microwave extraction method, aqueous extract gave positive results for the presence of phenols at different concentrations of methanolic kokum extract. Of these 100 % methanolic extract showed the maximum extraction of phenols followed by 80, 60, 40, and 20%. Fresh fruit showed highest concentration of phenols ie. 0.490 GAE/mg of sample as compared to dry rind and whole dry fruit.

Table 7.1.3: Phenolic Analysis of Extracts obtained using enzyme extraction

Conc. of methanol (%)	**Fresh fruit absorbance**	**GAE/mg of sample**	**Dry rind absorbance**	**GAE/mg of sample**	**Whole dry fruit**	**GAE/mg of sample**
100	0.393	**0.714**	0.332	0.599	0.283	0.510
80	0.232	0.426	0.183	0.330	0.163	0.294
60	0.130	0.239	0.112	0.202	0.180	0.195
40	0.126	0.227	0.110	0.198	0.102	0.183
20	0.125	0.225	0.108	0.194	0.101	0.182

By enzyme extraction method, aqueous extract gave positive results for the presence of phenols at different concentrations of methanolic kokum extract. Of these 100 % methanolic extract showed the maximum extraction of phenols followed by 80, 60, 40, and 20%. Fresh fruit showed highest concentration of phenols i.e. 0.714 GAE/mg **of** sample as compared to dry rind and whole dry fruit.

7.2. Comparative study of Adsorption residue on synthetic dye:

7.2.1 At varying p^H and concentration of adsorbent:

The effects of initial pH on dye solution of dyes removal were investigated by varying the p^H from 3 to 10. The removal of dye is maximum at stable p^H of dye.

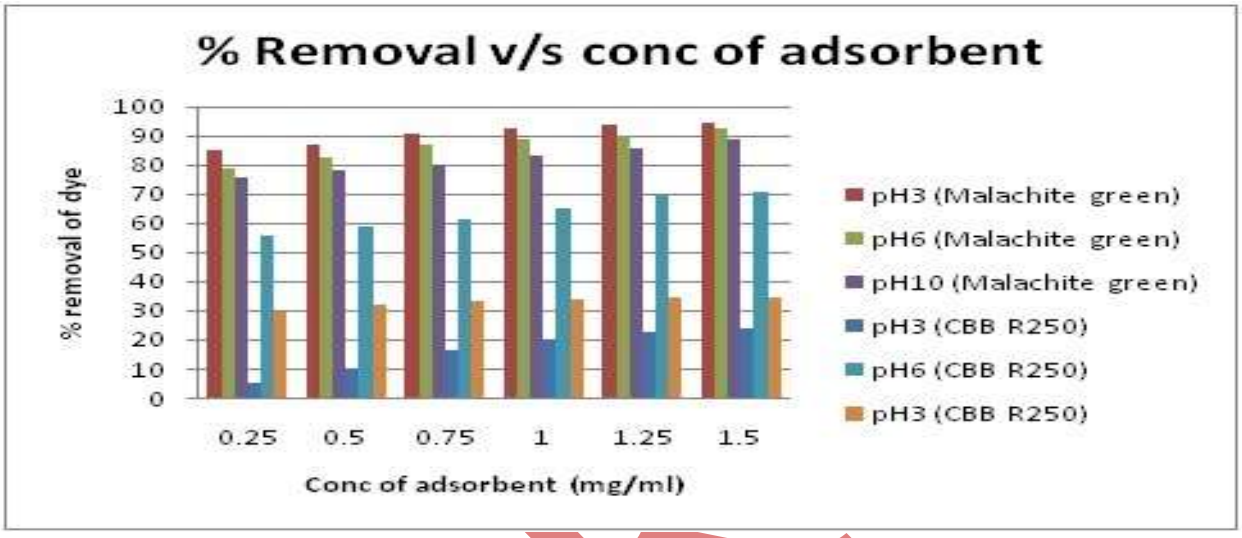

Fig 7.2.1: % Removal of dye v/s conc. of adsorbent (mg/ml)

For Malachite Green it was maximum at p^H = 3 as we see in the Fig 7.2.1. In case of methylene blue at p^H 6, greater is removal by adsorption .For Congo red and Nigrocine, there is no significant change in amount adsorbed after p^H 7. The adsorption of these dye is dependent on charge on dye as well as adsorbent surface.

7.2.2 At varying time and p^H of adsorbent:

The effect of contact time can be seen from Fig.7.2.2 for the dyes. It is clear that the extent of adsorption is rapid in the initial stages and becomes slow in later stages till saturation is allowed.

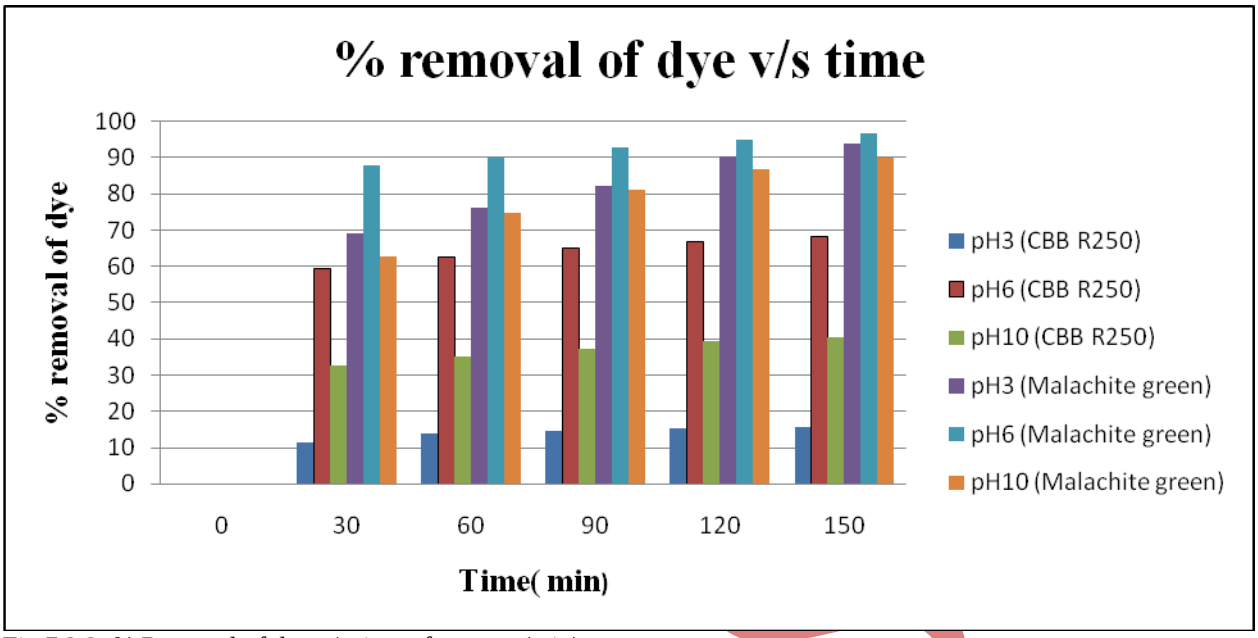

Fig 7.2.2: % Removal of dye v/s time of contact (min)

The final dye concentration did not vary significantly after 2 hours from the start of adsorption process. This shows that equilibrium can be assumed to be achieved after 2 hours (120 min). It is due to saturation of the active site which do not allow further adsorption to take place.

7.2.3 Results obtained from Freundlich and Langmuir Isotherm:

Langmuir Adsorption Isotherm describes quantitatively the formation of a monolayer adsorbate on the outer surface of the adsorbent, and after that no further adsorption takes place. The Langmuir isotherm is valid for monolayer adsorption onto a surface containing a finite number of identical sites. The model assumes uniform energies of adsorption onto the surface and no transmigration of adsorbate in the plane of the surface. Based upon these assumptions, Langmuir represented the following equation:

$$q_e = \frac{Q_0 K_L C_e}{1 + K_L C_e} \quad \text{---------- (3)}$$

In linear form,

$$\frac{1}{q_e} = \frac{1}{Q_0} + \frac{1}{Q_0 K_L C_e} \quad \text{---------- (4)}$$

Where: C_e = the equilibrium concentration of adsorbate (mg/L-1)

q_e = the amount of metal adsorbed per gram of the adsorbent at equilibrium (mg/g).

Q_o = maximum monolayer coverage capacity (mg/g)

K_L = Langmuir isotherm constant (L/mg).

The values of were computed from the slope and intercept of the Langmuir plot 1/qe versus 1/Ce.

The essential features of the Langmuir isotherm may be expressed in terms of equilibrium parameter R_L, which is a dimensionless constant referred to as separation factor or equilibrium parameter.

$$R_L = \frac{1}{1+(1+K_L C_e)} \quad \text{---------- (5)}$$

Where:

C_0 = initial concentration

K_L = the constant related to the energy of adsorption (Langmuir Constant).

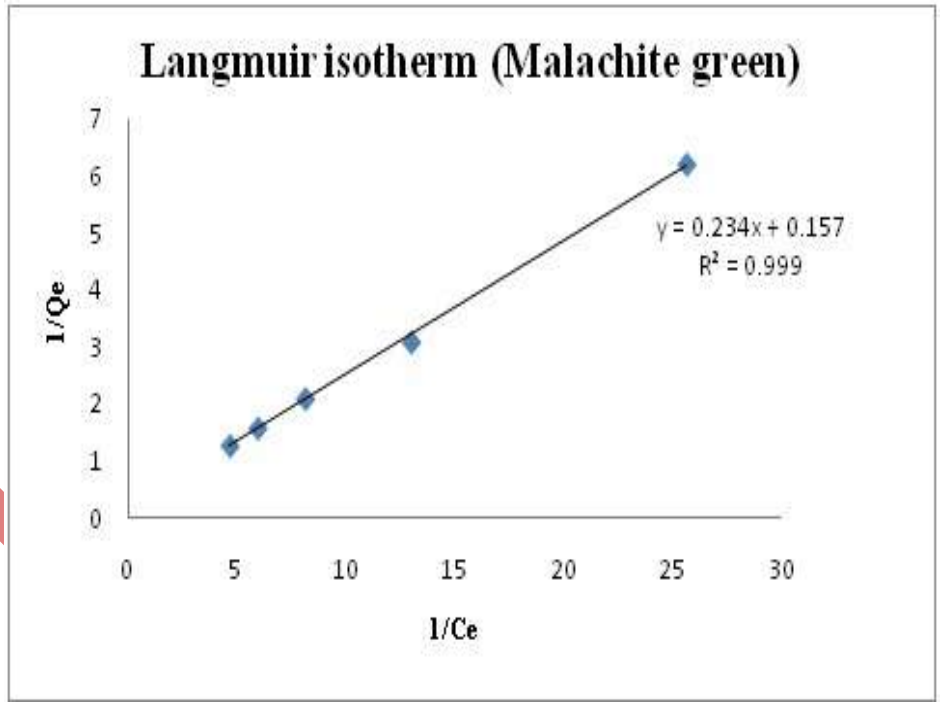

Fig.7.2.3.1: 1/Qe v/s 1/Ce

R_L value indicates the adsorption nature to be either unfavourable if $R_L>1$, linear if $R_L=1$, favourable if $0< R_L<1$ and irreversible if $R_L=0$.

Freundlich Adsorption Isotherm is commonly used to describe the adsorption characteristics for the heterogeneous surface. These data often fit the empirical equation proposed by Freundlich:

$$Q_e = K_f C_e^{1/n} \quad \text{---------- (6)}$$

Where

K_f = Freundlich isotherm constant (mg/g)

n = adsorption intensity;

C_e = the equilibrium concentration of adsorbate (mg/L)

Q_e = the amount of dye adsorbed per gram of the adsorbent at equilibrium (mg/g).

The constant K_f is an approximate indicator of adsorption capacity, while 1/n is a function of the strength of adsorption in the adsorption process.

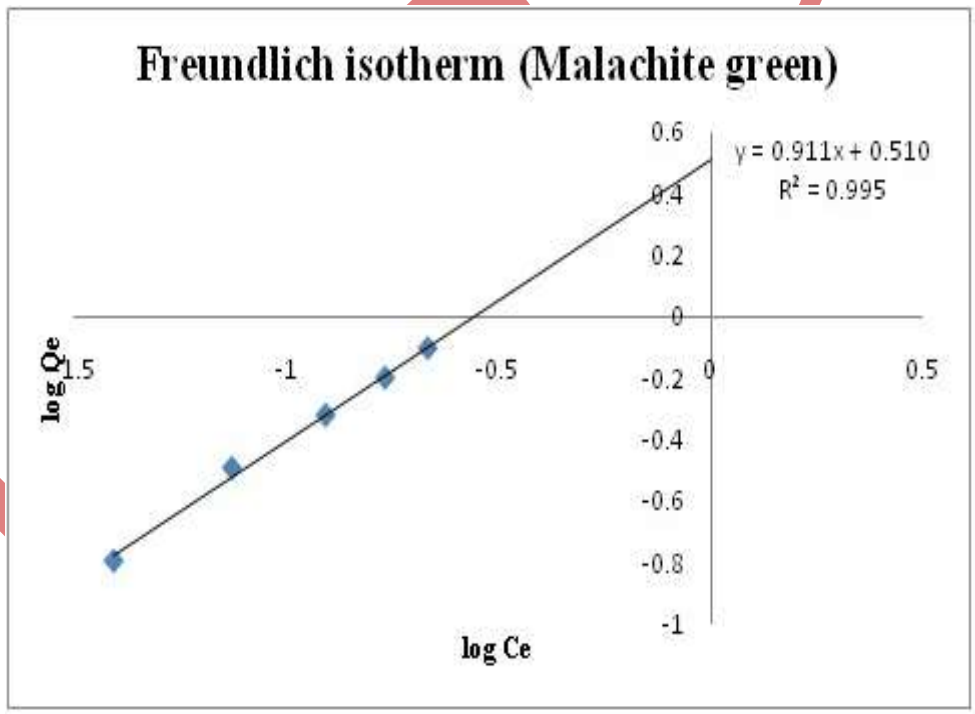

Fig. 7.2.3.2: log Qe v/s log Ce

If n = 1 then the partition between the two phases are independent of the concentration. If value of 1/n is below one it indicates a normal adsorption. On the other hand, 1/n being above one indicates cooperative adsorption.[12]

Table 7.2.3: Separation factor and adsorption intensity for dyes

Sr.no	Name of dye	Langmuir isotherm		Freundlich isotherm	
		RL (separation factor)	R^2 (regression)	1/n (adsorption intensity)	R^2 (regression)
1.	Commasie brilliant blue R-250 (CBB)	0.4	0.991	1.065	0.953
2.	Congo red	0.17	0.969	0.631	0.908
3.	Malachite Green	0.192	0.999	0.991	0.995
4.	Nigrocin	0.954	0.958	1.110	0.955

Here the value of 1/n is less than 1 for Malachite Green and Congo red indicating a normal adsorption and is greater than 1 for CBB and Nigrocin indicating cooperative adsorption. From the data calculated in above table the R_L is greater than 0 for all the dyes but less than 1 indicating that Langmuir isotherm is favourable for adsorbtion for all the dyes.

7.3. Results obtained on dyeing of clothes:

The dye obtained after concentrating of kokum peel extract was tested for its dyeing property on textile cloths like cotton, Teflon and wool using alum and vinegar as mordant in pre, post, and simultaneous mordanting method.

Fig 7.3: Dyeing of clothes using kokum dye extract

Results obtained from pre mordanting showed good dyeing property giving dark brownish color mainly to woolen cloth as shown in figure below Followed by post mordanting which gave less color to the cloths. Results were almost same for vinegar and alum with respect to colour intensity. However simultaneous mordanting did not show much coloring capacity.

7.4 Antimicrobial activity:

The antimicrobial activity of *Garcinia indica* peel extracts was studied against bacterial species like *Bacillus subtilis*, *Staphylococcus aureus*, *Escheresia coli.* The strains chosen are pathogenic and hence have harmful effects for human beings. It spreads diseases like diarrhoea, food poisoning, pneumonia etc.

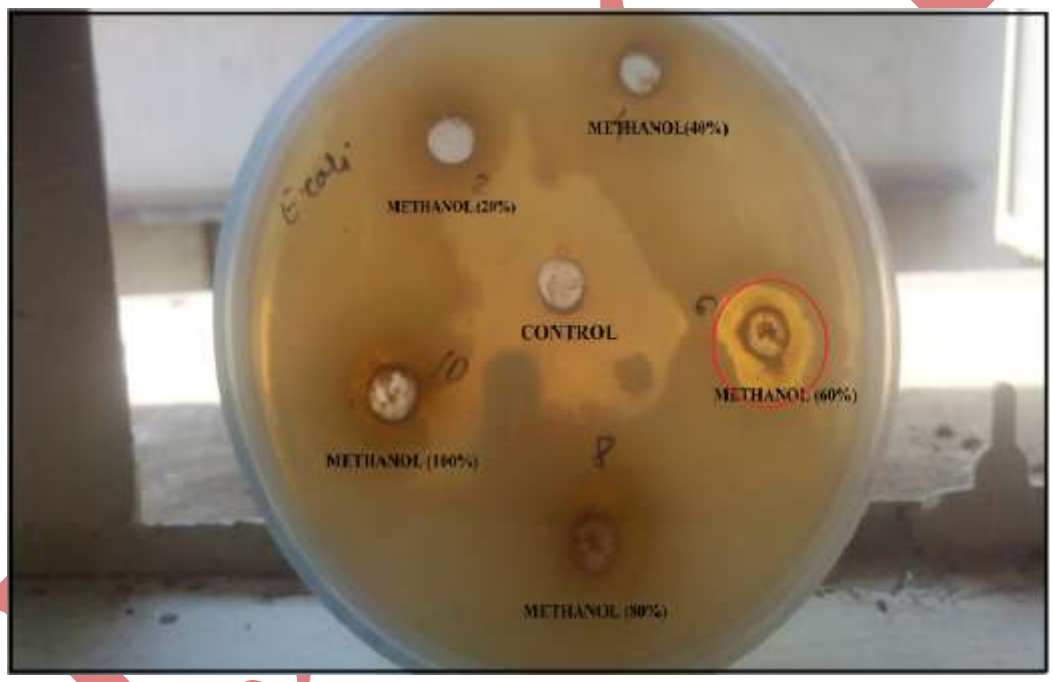

Fig. 7.4.1. Antimicrobial activity of *Garcinia indica* peel extract on *Escherichia coli*

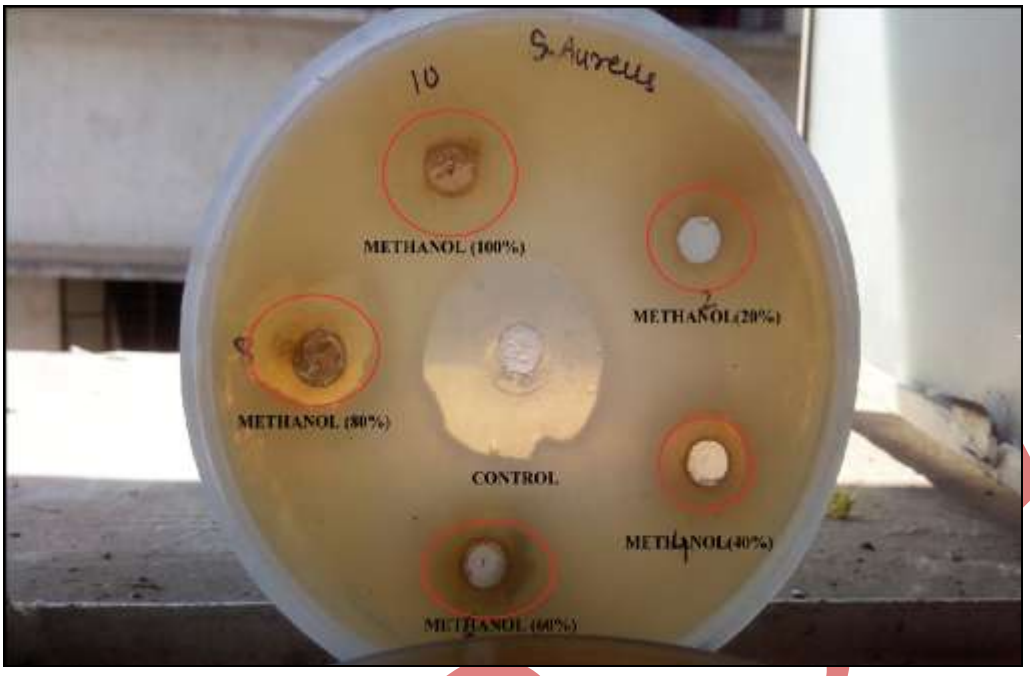

Fig. 7.4.2. Antimicrobial activity of *Garcinia indica* peel extract on *Staphylococcus aureus*

Fig. 7.4.3. Antimicrobial activity of *Garcinia indica* peel extract on *Bacillus subtulis*

For ultrasonication, the maximum antimicrobial activities were observed in 100% of methanol extract. For E.coli positive results were obtained for 60% methanolic extract. For Bacillus subtilis only 80 and 100% showed small

zone of inhibition. However *Staphylococcus aureus* showed maximum zone of inhibition for all concentrations of methanol extract especially for 80%. Hence it can be interpreted that Garcinia *indica plant* extract along with methanol can be used as an effective antimicrobial agent.

8. Conclusion:

The study on the *Garcinia indica* peel, fresh fruit and whole dry fruit has revealed the presence of phenols in it by phenols screening method. Many of them have good medicinal properties which are practically applicable for various clinical purposes. Comparing three methods of extraction, i.e. ultrasonication, enzyme assisted extraction and microwave extraction it has been seen that, enzyme assisted extraction method showed more yield for almost all the concentration of methanol. Also fresh fruit contains maximum concentration of phenols was found out by standard Gallic acid equivalent (GAE) assay. 100% of methanol concentration shows favorable condition for extraction

The residue left after filtering the extract was tested for its adsorption properties on removal of dyes like methylene blue, malachite green and Congo Red, Nigrocin from aqueous solutions by adsorption with the residue and has been experimentally determined and the following were observed: 1) The percentage of color removed increase with increasing adsorbent dosage, increases with increasing contact time and varied with dye solution pH. 2) Optimum contact time for equilibrium to be achieved is found to be 2 hours (120 min). It is basically due to saturation of the active site which do not allow further adsorption to take place. 3) For malachite green maximum adsorption found to be at pH = 9. In case of Methylene Blue higher the pH, greater is removal by adsorption. For Congo red there is no significant change in amount adsorbed after p^H 7. And Nigrocin showed no very little of adsorption.

Infact adsorption found to decrease with increase in pH of solution. The adsorption of these positively charged dye groups on the adsorbent surface is primarily influenced by the surface charge on the adsorbent which in turn is influenced by the solution pH. The adsorption data fitted into Langmuir and Freundlich isotherms of which Langmuir adsorption model was found to be have the highest regression value and hence the best fit. The value of the dimensionless separation factor, R_L was found to be <1 which confirms that the present adsorption system is favourable. The value of 1/n is below one it indicates a normal adsorption and above one indicates cooperative adsorption. It could be concluded that kokum rind residue is a potential and active biosorbent for removal of dye from its aqueous solution

Also dye extracted from kokum rind can be used in dyeing of cloths using various mordanting method.The extracts show antimicrobial properties in it which has been studied by antimicrobial activity assay. Furthermore from results it is confirmed that the plant extracts could be used for the treatment of various infections and superficial skin infections and it can be used as an effective antimicrobial agent.

9. Future Scope:

Extraction of phenols can be done using different solvents like ethanol, hexane, chloroform, propanol etc. having varying concentrations. Their yields using different extraction methods can be analyzed. Kokum residue can be further tested for its use in purification of waste water and removal of harmful metallic ions. Concentrated extract of kokum can be analyzed for its use in food color. It has scope in dyeing of textiles, so by optimizing various parameters it can be used in textile industries for dyeing purpose.

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