Experimental studies and Development of Modelling equation of Quercetin from *Raphanus Sativus* using Soxhlet Extractor

Meena Vangalapati[1], Gnana Madhuri Y[2], Vidya Sagar T[3], Ravi Raju V[4] and Satyadev L K[5]

[1.] Associate professor, Dept of chemical Engineering, AUCE (A), Andhra University, A.P, India

[2.] B.Tech. Student, Dept of Chemical Engineering, AUCE (A), Andhra University, A.P, India.

[3.] B.Tech. Student, Dept of Chemical Engineering, AUCE (A), Andhra University, A.P, India.

[4.] B.Tech. Student, Dept of Chemical Engineering, AUCE (A), Andhra University, A.P, India.

[5.] B.Tech. Student, Dept of Chemical Engineering, AUCE (A), Andhra University, A.P, India.

Abstract: The radish (*Raphanus sativus*) species belongs to Brassicaceae family. Different parts of radish including roots, seeds and leaves are used for medicinal purposes. The objective of this work was extraction of phenolic compounds from radish and to develop a modelling equation to describe quantitatively the extraction phenomena. The present study was intended to optimization of extraction of Quercetin and its various physico-chemical parameters have been studied. For the extraction of Quercetin the optimum results were observed for the effect of different solvents, soaking time, extraction time with hexane, particle size, different solvent percentages, different volumes of hexane with methanol as solvent and pH where the results showed up are respectively methanol, 1day, 1 hr, 200 particle mesh size, 80% (v/v), 1:1 ratio and 6.0. The highest Quercetin concentration for optimized condition was 45μg/ml. Soxhlet extraction was carried out using methanol for different extraction time periods to verify the mathematical model proposed in this work. The final form of proposed model was Es= $1.525(1-e^{-0.9033t})$ for Quercetin, where Es = yield extract (mg of quercetin/gm of dried sample) and t= extraction time (min). The model showed good agreement with the experimental data by generating average absolute relative deviation (AARD) of about 1.217+- 13.23% mg of quercetin per 1 gm of dried sample.

Key words: Radish, Quercetin, Soxhlet extractor, modelling equation, methanol

I. INTRODUCTION

The radish (*Raphanus sativus*) species belongs to Brassicaceae family and widely grown all over the world. *Raphanus sativus* L[1]. is originally from Europe and Asia. Different parts of radish including roots, seeds and leaves are used for medicinal purposes. The most popular part is the napiform tap root, although the entire plant is edible and the aerial parts (stem and leaf) can be used as leaf vegetables. The roots stimulate the appetite and digestion, having a tonic and laxative effect upon the intestines and indirectly stimulating the flow of bile. The juice of the fresh leaves is diuretic and laxative. The seed is carminative, diuretic, expectorant, laxative and stomachic. It is taken internally in the treatment of indigestion, abdominal bloating, wind, acid regurgitation, diarrhoea and bronchitis. The root is antiscorbutic , antispasmodic, astringent, cholagogue, digestive and diureti. It is crushed and used as a poultice for burns, bruises and smelly feet.. This specie is used popularly to treat liver and respiratory illnesses. The main constituents of radish are 4-(methylthio)-3-butenyl isothiocyanate, allyl isothiocayanate, benzyl isothiocyanate and phenethyl isothiocyanate, flavonoids [2] like quercetin and

antioxidants. It shows pharmacological activiyies like anti-fungal [3], anti-diabetic [4], anti-oxidant [5], anti-inflammatory[6] and anti cancer activities [7] etc.

II. MATERIALS AND METHODS

Chemicals and reagents:

Methanol, ethanol, ethyl acetate, distilled water, hexane, aluminium chloride, potassium acetate.

Equipment:

Soxhlet extractor, colorimeter, pH meter

Collection of the plant material:

The radish leaves are collected from local market at Visakhapatnam, Andhra Pradesh, India.

Processing of the plant material:

The leaves are cleaned, dried under shade and powdered. The powder is then screened in different mesh sizes (50,100,150,200) and the different fractions obtained are stored in an air-tight container.

Extract preparation:

2 gm of powder is added to 50 ml methanol (80 %) in a conical flask. The solution is soaked for 1 day. After the soaking time, the solution is filtered using Whatman filter paper no:1 and the filtrate is heated at 65 ^{o}C so that the solvent which is taken in glassware is evaporated. The resultant solution is cooled and make up to 25 ml with distilled water. This solution is added to 25 ml of hexane taken in a separating funnel. Incubate the solution of methanolic extract for 1 hr.

Determination of Quercetin

0.5 ml of methanolic extract is taken in a test-tube from the extract phase of the separating funnel. To this 1.5 ml of methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1M Potassium acetate and 2.8 ml of distilled are added. This mixture was allowed to stand for 30 min. The absorbance of the reaction mixture was measured at 450 nm using colorimeter. The concentration of Quercetin was determined by using calibration curve.

Solvent extraction using Soxhlet extractor

Prior to the solvent extraction study, 200 ml of 80 % methanol is taken in the extractor. 8 gm of dried radish leaf powder is placed in thimble and it is fixed to the condenser. Now the total apparatus is placed in the heater. Using the soxhlet apparatus continuous extraction is done for 5 hrs where 0.5 ml crude extract is collected for every 1hr for the determination of quercetin.

Modelling of Extraction of Quercetin using Soxhlet Extractor Apparatus

In order to describe the Quercetin transfer from *radish leaf* powder to the bulk of the solvent the following hypothesis were used. The mass transfer coefficient is constant. The solvent in the extractor is perfectly mixed, while the transfer resistance in the liquid phase is negligible and the Quercetin concentration in the solvent depends only on time. The transfer of the Quercetin was a diffusion phenomenon and independent of time. The final form of the modelling equation[8] was obtained from the extraction of Quercetin by using Soxhlet extractor[9].

$$E_s = B (1 - e^{-Dt})$$

where

Es = mg of Quercetin per gram of dried sample (mg/g),
t = extraction time (min) and B & D = equation constants.
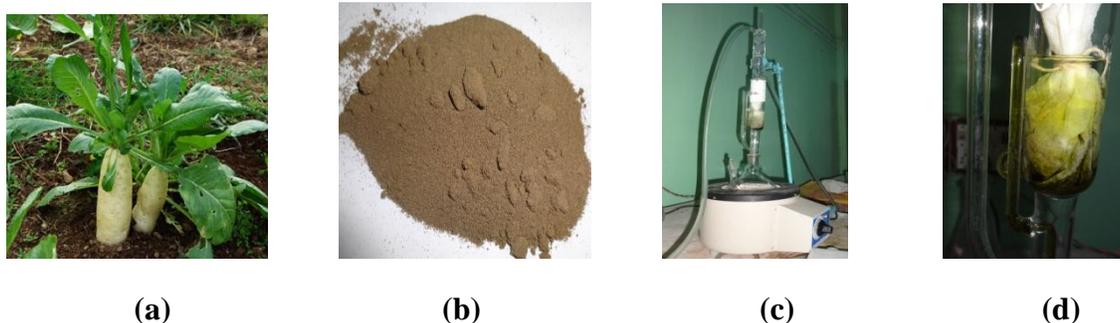
III. RESULTS AND DISCUSSION

| **(a)** | **(b)** | **(c)** | **(d)** |

Fig .1**:** (a) *Raphanus sativus* (b) Dried leaf powder (c) Soxhlet extractor (d) Quercetin extraction

A. EFFECT OF PROCESS PARAMETERS:

1. Effect of different solvents on extraction of quercetin :

For the extraction of Quercetin from radish leaf powder, different organic solvents such as methanol, ethanol, water and ethyl acetate are used. Among these, methanol showed the best results and the concentration of quercetin obtained was 16 μg/mL.(Fig. 2)
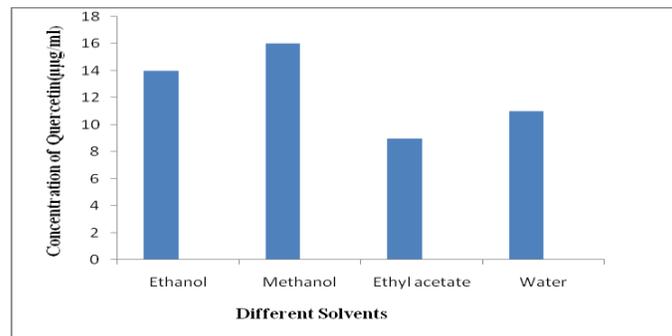
Fig. 2. Effect of different solvents on extraction of quercetin

2. Effect of soaking time on extraction of quercetin:

For the extraction of Quercetin from radish leaf powder, the samples were incubated for different time periods viz.,1 , 2, 3 and 4 days. It was observed that, soaking for 1 day gave the best result and the concentration of quercetin obtained was 20 μg/mL (Fig. 3)
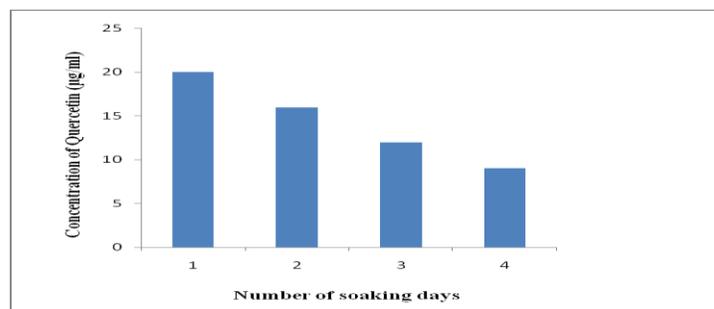

Fig. 3. Effect of soaking time on extraction of quercetin

3. Effect of extraction time with hexane on extraction of quercetin :

To investigate the influence of hexane on extraction of quercetin, different time intervals were taken viz., 1, 2,3 and 4 hours. Solvent- solvent extraction was done with hexane as one of the solvent. It was observed that, at the first hour, the concentration of quercetin was 25 μg/ml. (Fig. 4)
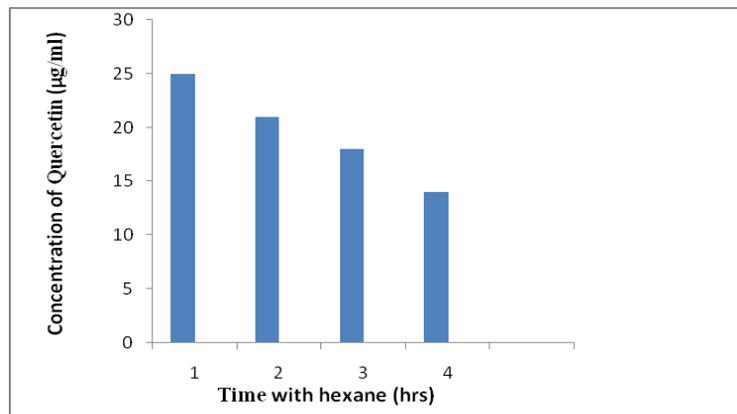
Fig. 4. Effect of extraction time with hexane on extraction of quercetin

4. Effect of differtent particle mesh sizes on extraction of quercetin:

Different particle mesh sizes viz., 50,100,150, 200 were used to find the optimum conditions for quercetin. In this investigation, the particles of 200 mesh size are found to be the optimum size for the quercetin extraction and quercetin's concentration is found to be 36 μg/ml.(Fig. 5)
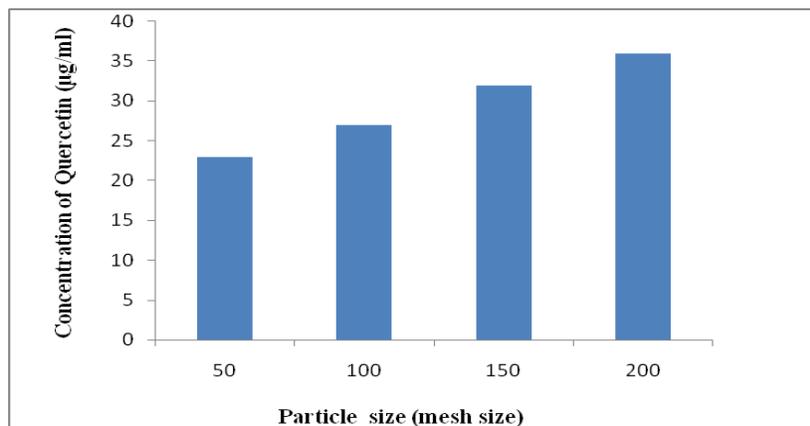

Fig. 5. Effect of differtent particle mesh sizes on extraction of quercetin

5. Effect of different solvent compostion on the extraction of quercetin:

Different solvent percentages like 0,20,40,60,80,100 are used for extraction of quercetin. The optimum solvent percentage was found to be 80% and its concentration is found to be 41 μg/ml.(Fig. 6)
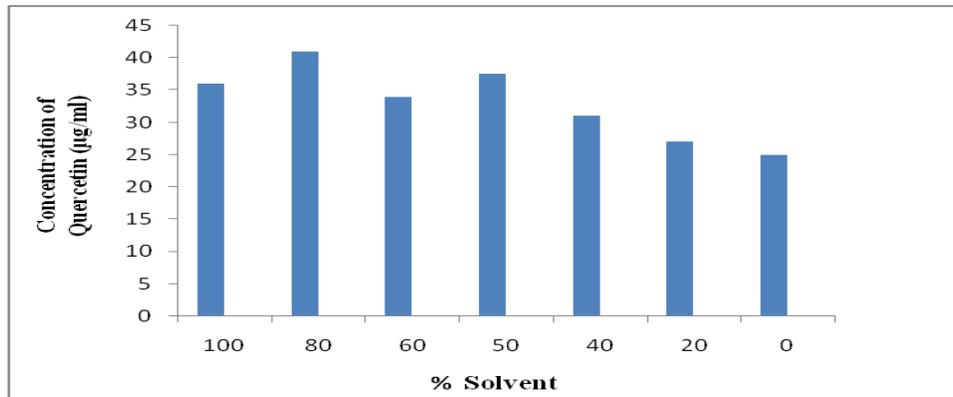
Fig. 6. Effect of different solvent compostion on the extraction of quercetin

6. Effect of different volumes of hexane on the extraction of quercetin:

Different volumes of hexane with solvent in different ratios such as 1:1, 1:2, and 2:1 are used for the extraction of quercetin. The optimum value is observed with the ratio 1:1 and the concentration of quercetin is found to be 41 μg/ml.(Fig. 7)
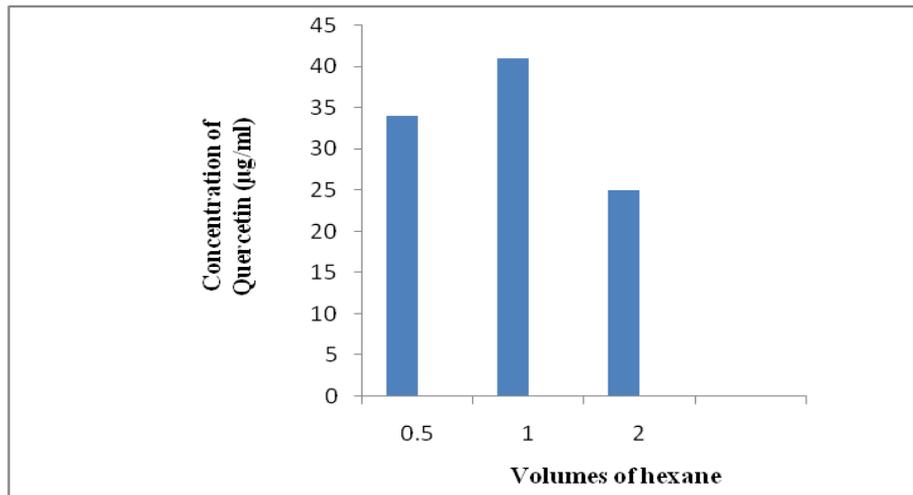

Fig. 7. Effect of different volumes of hexane on the extraction of quercetin

7. Effect of ph on extraction of quercetin:

To determine the effect of pH on extraction process different pH values namely, 4,5,6,7 and 8 were used. The optimum quercetin concentration of 45 μg/ml was observed at pH 6. (Fig. 8)
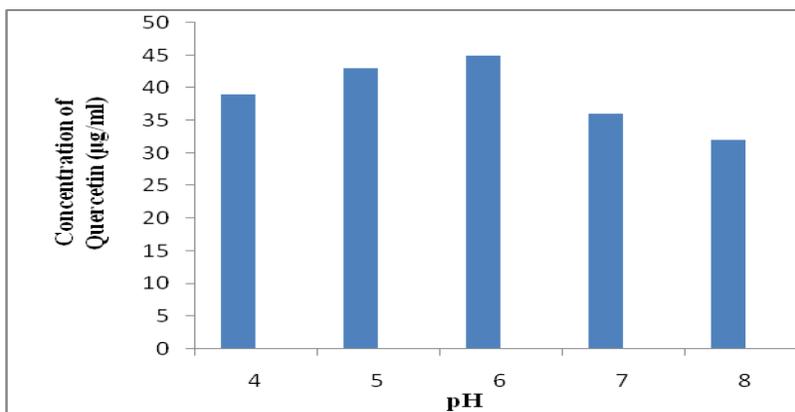
Fig. 8 Effect of ph on extraction of quercetin

B. RESULT OF MODELLING OF EXTRACTION OF QUERCETIN USING SOXHLET EXTRACTOR

In comparison to non- polar solvents, polar solvents could extract quercetin at higher yield except water, where hydrolysis and thermal degradation might occur. Non-polar solvents were almost unable to extract quercetin. Methanol was found to be the best solvent for the extraction of quercetin. The highest amount of quercetin extracted is from 80% methanol concentration solution. The final form of the proposed model equation for quercetin was

$$E_s = 1.525(1-e^{-0.9033t})$$

where Es = yield extract (mg of quercetin/gm of dried sample and t= extraction time (min). The model showed good agreement with the experimental data by generating average absolute relative deviation (AARD) of about 1.217+- 13.23% mg of quercetin per 1 gm of dried sample.

Table : Effect of Yield extract with extraction time on Quercetin: (Fig 9)

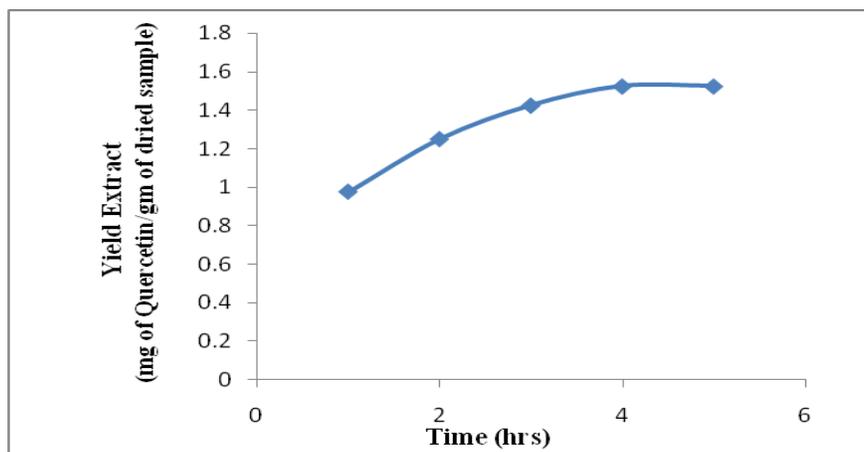| S.No	Time (hr)	Yield extract (mg of Quercetin/gm of dried sample)
1	1	0.975
2	2	1.25
3	3	1.425
4	4	1.525
5	5	1.525

Fig. 9 Effect of Yield extract with extraction time on Quercetin

IV. CONCLUSION

The present study was intended for the optimization of extraction of Quercetin and its various physico- chemical parameters have been studied. For the extraction of Quercetin the optimum results were observed for the effect of different solvents, soaking time, extraction time with hexane, particle size, different solvent percentages, different volumes of hexane with methanol as solvent and pH were methanol, 1day, 1 hr, 200 particle mesh size, 80% (v/v), 1:1 ratio and 6.0 respectively. The highest Quercetin concentration for optimized condition was 45µg/ml.

Methanol was found to be the best solvent for the extraction of Quercetin from radish by soxhlet extractor. This Solvent extraction using soxhlet extractor was conduct to verify the mathematical model proposed in this work. The final form of proposed models were Es= $1.525(1-e^{-0.9033t})$ for Quercetin, where Es = yield extract (mg of quercetin/gm of dried sample and t= extraction time (min). The model showed good agreement with the experimental data by generating average absolute relative deviation (AARD) of about 1.217 +_ 13.23 % mg of quercetin per 1 gm of dried sample.

REFERENCES

[1] Rosa Martha Perez Gutierre and Rosalinda Lule Perez, "*Raphanus sativus* (Radish): Their Chemistry and Biology", The Scientific World Journal. Vol.4: pp 811–837, 2004.
[2] Ali Ghasemzadeh, Maryam Azarifar, Omid Soroodi and Hawed Z. E. Jaafar, " Flavonoid compounds and their antioxidant activity in extract of some tropical plants", J. Medicinal Plants Research.Vol. 6(13): pp 2639-2643, 2012.
[3] Aerts AM, Francois IE, Meert EM, Li QT, Cammue BP and Thevissen K, "The anti-fungal activity of RsAFP2, a plant defensin from Raphanus sativus, involves the induction of reactive oxygen species in Candida albicans", J Mol Microbial Biotechnol. Vol.13(4): pp 243-247, 2007.
[4] Farzaneh Dehghani, Monireh Azizi and Mohammad Reza Panjeshahin, "The effects of aqueous extract of Raphanus sativus on blood glucose, tri-glyceride and cholesterol in diabetic rats", Iranian Journal of Pharmacology and Therapeutics. Vol.10: pp 66-70, 2011.
[5] Jalila Ben Salah-Abbès Samir Abbès, Zouhour Ouanes, Zohra Houas, Mosaad A. Abdel-Wahhab,
Hassen Bacha and Ridha Oueslati ,"Tunisian radish extract (*Raphanus sativus)* enhances the antioxidant status
and protects against oxidative stress induced by zearalenone in Balb/c mice", *J. Appl.Toxicol*.Vol 28:pp 6–14,2008.
[6] Shobha Kamble, Md. Zubair Ahmed, S.Ramabhimaiaha and Prabhakar Patil, "Anti-Inflammatory activity of Raphanus sativus L in Acute and Chroniv Experimental Models in Albino Rats", Biomedical and Pharmacology Journal. Vol.6(2):pp 315-320, 2013.
[7] Mohamed A. Abd-Elmoneim, Ashraf A. Bakar, Isis M Awad, Ehab M. Mohamed and Sorial A. Moharib, "Anticarcinogenic effect of Raphanus sativus on 1,2-Dimethylhydrazine(DMH) Induced Colon Cancer in Rats", The Egyptian Journal of Hospital Medicine. Vol.51:pp 473-486, 2013.

[8] D V Surya Prakash, N Sree Satya and Meena Vangalapati, "Extraction of Chebulinic acid fromTerminalia Chebula sp. Soxhlet extractor - An experimental and modeling studies", Asian Journal of Biochemical and Pharmaceutical Research.Vol.2(3): pp 170-176, 2012.

[9] G.P. Choudhary, "Wound healing activity ethanolic extract of Terminalia Chebula ", International Journal of Pharma and Biosciences.Vol. 2(1), pp 48-52, 2011.

BIOGRAPHY

Dr.Meena Vangalapati M.Tech., Ph.D, Associate Professor, Department of Chemical Engineering, Andhra University College of Engineering (A), Andhra University, Visakhapatnam, Andhra Pradesh, India. She has 13years of   teaching experience and   published 60 more Papers published in National and International Reputed Journals, published 7 monographs/Books.
E-mail id : meena_sekhar09@yahoo.co.in.

Gnana Madhuri .Y B.Tech, Department of Chemical Engineering,
Andhra University College of Engineering (A), Andhra University, Visakhapatnam, Andhra Pradesh,India.   She has attended 4 more National and International conferences.
e-mail id : y.gnanamadhuri@gmail.com.

Vidya Sagar .T B.Tech, Department of Chemical Engineering,
Andhra University College of Engineering (A), Andhra University, Visakhapatnam, Andhra Pradesh,India. He has attended 6 more National and International conferences. He has 1paper   published in International Reputed Journal.
e-mail id : thumatividyasagar@gmail.com.

Ravi Raju. V B.Tech, Department of Chemical Engineering,
Andhra University College of Engineering (A), Andhra University, Visakhapatnam, Andhra Pradesh,India. He has attended 6 more National and International conferences.
e-mailid:ravirajuvysyaraju2@gmail.com

Satyadev L .K, B.Tech, Department of Chemical Engineering,
Andhra University College of Engineering (A), Andhra University, Visakhapatnam, Andhra Pradesh,India. He has attended 5 more National and International conferences.
e-mailid:prasadlakkimsetti1@gmail.com