Combined Antibacterial Activity of Medicinal Plant Extracts with Metal Salts

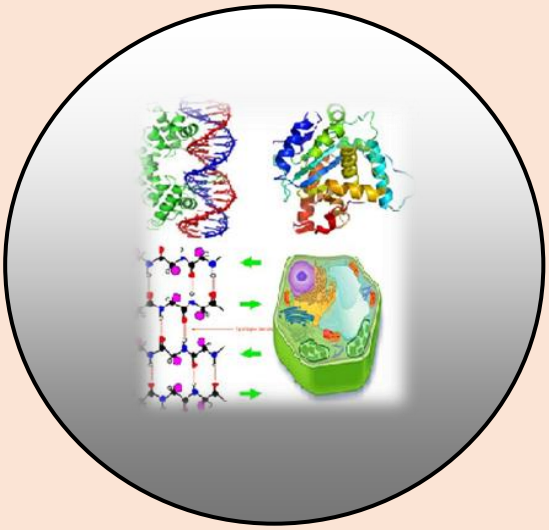
By

Gezahegn Faye, Melkamu Fayera, Shifera Demissie and Dale Abdisa

ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

Index Copernicus International Value
IC Value of Journal 82.43 Poland, Europe (2016)
Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66

J. Biol. Chem. Research
Volume 37 (1) 2020 Pages No. 120-126



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 37, No. 1: 120-126, 2020
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 37/01/1024/2020

ISSN 2319-3077 (Online/Electronic)
ISSN 0970-4973 (Print)

http:// www.sasjournals.com
http:// www.jbcr.co.in
jbiolchemres@gmail.com

RESEARCH PAPER

Received: 13/01/2020 **Revised: 11/03/2020** **Accepted: 12/03/2020**

Combined Antibacterial Activity of Medicinal Plant Extracts with Metal Salts

***Gezahegn Faye, Melkamu Fayera,**
****Shifera Demissie and Dale Abdisa**

Department of Chemistry, College of Natural Sciences, Jimma University, P.O. Box 378, Jimma, Ethiopia
***Department of Chemistry, College of Natural Sciences, Salale University, P.O. Box 245, Fiche, Ethiopia**
****Department of Biology, College of Natural Sciences, Jimma University, P.O. Box 378, Jimma, Ethiopia**

ABSTRACT

Kniphofia pumila, Rhamnus staddo A. Rich and Clutia abyssinica have been reported to have antibacterial activities and the community of Oromia regional state, western part of Ethiopia are also using for the treatment of different diseases. The main objective of this study was to perform extraction of compounds from these plants and antibacterial activity study in combination with metal salts.

Cold maceration technique was used for extraction of compounds from root part of *K. pumila,* steam bark of *R. staddo A. Rich* and *C. abyssinica* sequentially with petroleum ether, chloroform, acetone and methanol. The nature of electronic transition in crude extracts, and combined extract with metal salts were characterized by UV-vis spectroscopic. The *in vitro a*ntibacterial activity study of crude extract and combined crude extract with metal salts were conducted by disc diffusion method against *E.coli* (ATCC 25722), *K. pneumonia* (DSM 19613), *S. aureus* (ATCC 25925) and *S. typhimurium* (ATCC 13311).

Generally, the antibacterial activities of the crude extracts observed were lower except for *R. staddo A. Rich* showed better zone of growth inhibition against *S. aureus* (ATCC 25925). Whereas, the combined crude extract with metal salts displayed antibacterial activities, even in some cases zone of growth inhibition greater than the standard drug were recorded.

The study validated novel and promising antibacterial activity of combined crude extract with metal salts, especially *R. staddo A. Rich* PeZn, *K. pumila* AcZn and *C. abyssinica* AcZn showed remarkable growth inhibition zone of 20 ±0.20 mm against *K. pneumonia*, 25 ± 0.44 mm against *K. pneumonia* and 26 ± 0.18 mm against *S. typhimurium* respectively.

Keywords: medicinal plants, antibacterial activity, metal salt, maceration method

INTRODUCTION

Infectious diseases by microorganisms are causes for the annual death of millions of people (Subhasish S. *et al*., 2009) and are worldwide problem. It has been mentioned that the increasing rate of multiple drug resistance by microbial infections

(Edilu A., *et al*., 2015; Prescott H and Klein JO, 2002), and the slowed down rate of new drug discovery to tackle them[3] makes the diseases to be widespread and challenging. For example, methicillin-resistant *S.aureus* (MRSA) and vancomycin-resistant *enterococcus* (VRE) (Graham L. P., 2013) which were treatable pathogens and are now untreatable. Medicinal plants contain bioactive phytochemical constituent such as alkaloids, saponins, glycosides, flavonoids, phenol, coumarins, terpenes and carboxylic acids that provide antimicrobial properties to the plant (Neelam R. *et al*., 2016). Traditional medicinal plants used by human being as an antimicrobial, anticancer, anti-inflammatory, antidiabetic, antioxidant and antidiuretic activities are therefore mainly depends on these constituents. Natural products provide many new chemical structures, which no chemist would dream of synthesizing. For example, the anti-malarial drug artemisinin is one such example, containing an extremely unstable-looking trioxane, ring-one of the most unlikely structure to have appeared in recent years (Tehmina S. *et al*., 2013). Medicinal plants have been also reported to be good sources for coordinating ligand compounds such as curcumin, morin, quercetin and nicotine that chelate metal ions, and the antimicrobial activities of their complexes were reported to be greater than the free ligand (Subhasish S. *et al*., 2009; Erin M McC.*et al.,* 2008; Prabhjot S. J. *et al.,* 2015; Prashanth MK *et al.,* 2013). Similarly, different scholars also reported enhanced antimicrobial activities of combined crude extracts and metal salt when compared with pure crude extracts (Subhan M. A. *et al*., 2014; Safana A. F. *et al*., 2013; Antonio F. S. *et al*., 2014). However, very limited study report found on combined antibacterial activity of crude extract with metal salts, and there is no literature report on the combined antibacterial activity study of metal salts with extracts from *K. pumila, R. staddo A. Rich and C. abyssinica.* The societies in the study area are using these medicinal plant parts for curing different diseases. Thus, this research focused on antibacterial activity investigation of crude extracts of *K. pumila, R. staddo A. Rich and C. abyssinica* along with metal salts against selected food borne pathogens to design new approach to combat drug resistant food borne bacterial infections.

MATERIALS AND METHOD

Study area and period

The sample was collected in the month of November, 2017 from Oromia regional state, western part of Ethiopia, and the study carried out in Jimma University, located at 353 km to the southwest of Addis Ababa. The herbarium of the plants were identified and deposited at Jimma University, Department of Biology, Research and Postgraduate Laboratory. The phytochemical screening conducted at Chemistry Laboratory, and the antimicrobial susceptibility testing at Research and Postgraduate Laboratory, Department of Biology, Jimma University.

Chemicals and Apparatus

Sigma-Aldrich analytical grade reagent (AR) chemicals such as petroleum ether, chloroform, acetone, methanol dimethylsulfoxide (DMSO), $CuCl_2$, $NiCl_2$, $ZnCl_2$, NaOH, Potassium ferricynide, Potassium Iodide, Glacialacetic acid, HCl, H_2S0_4, Dragenroff's, Nutrient Broth, Nutrient agar and Muller Hinton Agar as culture medium, and gentamycin; apparatus such as rotary vapor (Model), column chromatography, round bottom flask, electrical balance, volumetric flask, measuring cylinder, pestle and mortar, filter papers, weighing balances, water bath, reflux condenser, Thermometer and UV-Vis spectrophotometer (Model 6705 JENWAY) were used.

Plant material Collection and preparation

The fresh plant materials, steam bark of *R. staddo A. Rich and C. abyssinica*, root part of *K. pumila,* were collected from Oromia regional state, western part of Ethiopia. The collected plant materials were chopped into smaller pieces and shade dried in laboratory at room temperature without any exposure to direct sunlight. The dried plant materials then powdered to facilitate easy solvent penetration and stored in suitable containers until used for extraction.

Extraction of the plant material

One kilogram of each powdered plant materials were sequentially extracted with an equal volume of about 2.5 liter petroleum ether, chloroform, acetone and methanol using maceration technique for 24 hrs with shaking. The extracts of each solvent was filtered first through a fresh cotton plug and then through fluted filter paper. The filtrates were concentrated by evaporation under reduced pressure using a Rota vapor at 40 ^{o}C. The resulting semidried extracts of each solvents were weighed and stored in refrigerator below 4^{o}C until used for antibacterial assay.

Antibacterial assay using the agar diffusion method

The antibacterial activity tests of the samples against food born bacterial pathogens such as *E. coli (*ATCC 25722*), K. pneumonia* (DSM 19613), *S. aureus* (ATCC 25925) and *S. typhimurium* (ATCC 13311) were conducted using agar disc diffusion method as described in Mackeen *et al.* (1997) and results were interpreted as per the criteria of the National Committee for Clinical Laboratory Standards (NCCLS, 2007). Bacterial strains cultures were first grown on agar plates at 37°C for 24hrs prior to inoculation on to the nutrient agar. The 6 mm diameter sterile discs of what man No3 paper was placed on the surface of the inoculated agar approximately at equal distance of corners in petri plates in a 200 mg/mL concentration that were prepared by dissolving 200 mg of crude extracts in 1 mL of DMSO. 50mg/mL test solution of $NiCl_2$, $CuCl_2$ and $ZnCl_2$ were also prepared in separate volumetric flask. Whereas, the combined crude extracts and these metal salts solutions were prepared by mixing their solutions in 1:1 ratio with stirring.

Then, the test solutions were added on the discs, allowed to diffuse for 5 minutes and the petri plates kept in an incubator at 32°Cfor 24 hrs. The antibacterial activity resulted was recorded after 24 hrs by measuring the diameter of growth inhibition on the discs (in mm) using transparent ruler. Standard for anti-bacterial drug (gentamycin) was used as positive control and DMSO as negative control. The bacterial strains were obtained from Jimma University, Biology Department.

RESULT AND DISCUSSION

Uv-visible Spectroscopic study

The Uv-visible spectra of *K. pumila*, *R. staddo A. Rich and C. abyssinica* extract showed characteristics peaks which can be assigned π→π* and n→π*electronic transition (Yevgen P. *et al.*, 2005). Similarly, the Uv-visible spectra of the combined metal ions and extracts showed all the characteristic peaks with blue shift or red shift when compared with the wavelength values measured for extracts alone. In very few cases, the disappearances of peaks of the extracts were also observed. The shift in wavelength and the peak disappearances of combined extract and metal salt compared to that of the extracts are indicators for the bonding of functional groups of the extracts with metal ions i.e. complexation of metal with extracts. The binding of metal ions by extracts were also confirmed by change of colors upon mixing and stirring the solutions of reactants, metal ions and extracts. The shift in absorption maxima of the extracts upon combination with metal ions, and formation of new colors for combined metal ions and extracts are indicators for the participation of extracts in coordination with the metal ions (Muhammad I. Z. *et al.*, 2012).

Phytochemical analysis

Phytochemical screening tests for alkaloids, flavonoids, phenols, quinines and terpenoids were conducted in all extracts using standard procedures described by Harborne (1973) and Trease and Evans (1989). Generally, the result revealed that the extracts of *C. abyssinica*, *R. staddo A. Rich* and *K. pumila* contain alkaloids, phenols, quinines and terpenoids (Table 1-3). Alkaloids were detected in both petroleum ether and chloroform extracts; phenols, terpenoids and quinones were found in solvents extracts of petroleum ether, chloroform, acetone and methanol in the three plants, and thus the presence these secondary metabolites in the plants is supportive evidence for the tradition use clam of the plants by the society.

Table 1. Phytochemical analysis of steam bark extracts of *C. abyssinica.*

Plant constituents	Petroleum ether	Chloroform	Acetone	Methanol
Alkaloids	+	+	-	-
Flavonoids	-	-	-	-
Phenols	-	-	-	+
Quinones	-	-	-	+
Terpenoids	-	+	+	-

- *not present* **+** *present*

Table 2. Phytochemical analysis of steam bark extracts of *R. staddo A. Rich.*

Plant constituents	Petroleum ether	Chloroform	Acetone	Metanol
Alkaloids	-	+	-	+
Flavonoids	-	-	-	-
Phenols	+	+	+	+
Quinones	+	+	+	+
Terpenoids	-	-	+	+

Table 3. Phytochemical analysis of root extracts of *K. pumila.*

Plant constituents	Petroleum ether	Chloroform	Acetone	Metanol
Alkaloids	+	-	-	-
Flavonoids	-	-	-	-
Phenols	-	-	+	+
Quinones	+	+	+	+
Terpenoids	+	+	+	+

Table 4: **Antibacterial Activity results for extracts.**
Diameter of inhibition zone (mm) (Mean ± S.D)

Bacteria strains	*R. staddo A. Rich*				*K. pumila*				*C. abyssinica*			
	Me	**Ch**	**Ac**	**Pe**	**Me**	**Ch**	**Ac**	**Pe**	**Me**	**Ch**	**Ac**	**Pe**
E. coli	6.11± 0.62	NI	7.51± 0.67	NI	NI	9.7± 0.38	12.6± 0.39	NI	NI	8.31± 0.43	NI	NI
S. typhimurium	NI	6.91± 0.71	NI	7.23± 0.19	NI	NI	9.7± 0.28	NI	8.10± 0.56	NI	6.77± 0.24	NI
S. aureus	15± 0.23	16± 0.14	16± 0.16	15± 0.34	10.7± 0.39	10.8± 0.45	10.7± 0.32	12± 0.45	NI	6.97± 0.41	NI	6.35± 0.35
K. pneumonia	7.8± 0.35	NI	7.67± 0.23	NI	NI	NI	11.8± 0.41	NI	7.88± 0.37	NI	7.95± 0.22	NI

***Me**-methanol extract, **Ac**- acetone extract, **Pe**-petroleum extract, **Ch**-chloroform extract, **NI**-no inhibition*

Table 5. Antibacterial activity of metal salts, Gentamycin and DMSO.

Bacterial strains	Diameter of inhibition zone (mm)) (Mean ± S.D)				
	$CuCl_2$	**$ZnCl_2$**	**$NiCl_2$**	**Gentamycin**	**DMSO**
E.coli	16±0.19	17±0.18	15±0.20	16±0.19	NI
S. typhimurium	16±0.20	16±0.16	10±0.21	15±0.15	NI
S. aureus	9±0.17	14±0.18	13±0.23	13±0.17	NI
K. pneumonia	12±0.16	17±0.19	12±0.19	18±0.25	NI

Antibacterial Activity Study

The antibacterial activity of the tested samples was assessed by the diameter of zone of inhibition in millimeters and zones of inhibition more than 6 mm were recorded. In general, the antibacterial activity of the crude extracts from the three plants showed lower antibacterial activities in some cases no growth of inhibition observed (Table 4). However, *R. staddo A. Rich* showed better antibacterial activity towards one strain, *S. aureus.* Whereas, the combined extracts with metal salts showed positive results against all bacterial strains except *R. staddo A. Rich* Ch Cu against *E. coli and R. staddo A. Rich* ChNi against *S. aureus* showed no zone of growth inhibition (Table 5-8).

Some combined extracts with metal salts showed higher activity than the standard positive control drug, gentamycin. Generally, the antibacterial activity of the combined extracts with zinc salt was observed to be promising in all the three plant considered, especially *R. staddo A. Rich* PeZn, *K. pumila* Ac Zn and *C.abyssinica* AcZn showed a growth inhibition zone of 20±0.20 mm against K. *pneumonia*, 25 ± 0.44mm against *K .pneumonia* and 26 ± 0.18mm against *S. typhimurium* respectively. The enhanced antibacterial activities of combined extracts with metal salts are likely due to the effect of metal ions (Tehmina S. *et al.*, 2013; Simon WJ G. *et al.*, 2009). Which means the metal ions may combine with chemical constituents of the extracts to form complex compounds and many of these chemical constituents (Table 1) have been reported to have complexation properties with metal ions and thus, show enhanced activity as result of lipophilic property (Antonio F. S. *et al*., 2014; Muhammad I. Z. *et al.*, 2012).

Table 6. Antibacterial Activity of combined *R. staddo A. Rich* extracts-metal salts.

Bacteria strains	Diameter of inhibition zone (mm)) (Mean ± S.D)											
	AcCu	**AcZn**	**AcNi**	**PeCu**	**PeZn**	**PeNi**	**ChCu**	**ChZn**	**ChNi**	**MeCu**	**MeZn**	**MeNi**
E.coli	7± 0.22	12± 0.29	9± 0.55	8± 0.27	16± 0.19	12± 0.45	NI	21± 0.31	6± 0.55	18± 0.63	15± 0.50	9± 0.51
S.typhimurium	10± 0.19	16± 0.33	10± 0.50	9± 0.23	13± 0.21	11± 0.38	15± 0.37	11± 0.27	10± 0.45	10± 0.53	16± 0.56	7± 0.63
S.aureus	15± 0.25	16± 0.30	15± 0.47	16± 0.30	15± 0.22	11± 0.41	16± 0.45	13± 0.29	13± 0.60	24± 0.49	18± 0.48	17± 0.52
K..pneumonia	8± 0.21	12± 0.29	6± 0.60	10± 0.25	20± 0.20	11± 0.57	10± 39	12± 0.30	11± 0.50	6± 0.54	10± 0.53	6± 0.56

Table 7. Antibacterial Activity of combined *K. pumila* extracts-metal salts.

Bacteria strains	Diameter of inhibition zone (mm)) (Mean ± S.D)											
	AcCu	**AcZn**	**AcNi**	**PeCu**	**PeZn**	**PeNi**	**ChCu**	**ChZn**	**ChNi**	**MeCu**	**MeZn**	**MeNi**
E. coli	19± 0.44	12± 0.23	10± 0.27	18± 0.51	23± 0.34	6± 0.45	8± 0.47	21± 0.53	7± 0.31	8± 0.66	20± 0.61	6± 0.56
S. typhimurium	7± 0.29	12± 0.28	6± 0.33	21± 0.43	10± 0.44	6± 0.46	10± 0.51	11± 0.41	11± 0.28	8± 0.47	18± 0.59	10± 0.61
S. aureus	9± 0.32	11± 0.31	8± 0.25	8± 0.65	11± 0.29	6± 0.39	8± 0.49	15± 0.57	8± 0.26	9± 0.52	14± 0.58	11± 0.59
K. pneumonia	20± 0.41	25± 0.44	13± 0.28	15± 0.37	20± 0.42	11± 0.42	20± 0.53	20± 0.33	8± 0.29	15± 0.63	18± 0.72	13± 0.67

Table 8. Antibacterial Activity of combined *C.abyssinica* extracts-metal salts.

Bacteria strains	Diameter of inhibition zone (mm)) (Mean ± S.D)											
	AcCu	**AcZn**	**AcNi**	**PeCu**	**PeZn**	**PeNi**	**ChCu**	**ChZn**	**ChNi**	**MeCu**	**MeZn**	**MeNi**
E .coli	9± 0.16	20± 0.17	12± 0.18	11± 0.21	20± 0.24	10± 0.31	10± 0.39	11± 0.21	8± 0.31	9± 0.43	11± 0.38	15± 0.51
S. typhimurium	15± 0.22	26± 0.18	12± 0.21	10± 0.18	16± 0.19	12± 0.29	16± 0.28	12± 0.33	11± 0.34	10± 0.41	15± 0.45	7± 0.49
S. aureus	10± 0.20	20± 0.15	10± 0.19	8± 0.23	10± 0.21	10± 0.21	9± 0.31	13± 0.28	NI	12± 0.50	10± 0.50	10± 0.47
K. pneumonia	20± 0.19	25± 0.20	13± 0.15	15± 0.19	20± 0.25	11± 0.31	20± 0.40	20± 0.41	8± 0.37	15± 0.44	18± 0.51	13± 0.50

CONCLUSION

Traditionally claimed medicinal plants, root of *K. pumila, steam bark of R. staddo A. Rich and C. abyssinica,* have been used in Oromia regional state, western part of Ethiopian society to alleviated different diseases. Fresh plant materials were collected; dried and sequential extraction with petroleum ether, chloroform, acetone and methanol using maceration technique has been performed.

The *in vitro* antibacterial assay was performed by disc diffusion method, and among the crude extracts of the three plants, *R. staddo A. Rich* revealed better antibacterial activities against *S. aureus* (ATCC 25925). However, the observed antibacterial activities of almost all combined crude extract-metal salts were promising against *E. coli (*ATCC 25722*), K. pneumonia* (DSM 19613), *S. aureus* (ATCC 25925) and *S. typhimurium* (ATCC 13311). The antibacterial activity of combined extracts with zinc salt specifically *R. staddo A. Rich* PeZn, *K. pumila* AcZn and *C. abyssinica* AcZn showed remarkable growth inhibition zone against *K. pneumonia*, *K. pneumonia* and *S. typhimurium* respectively. Hence, combined extracts of these plants and zinc salt could be the potential candidate for the future antibacterial drug development.

ACKNOWLEDGMENTS

All the activities (designing, data collection, analysis and interpretation) under this research work has been fully financed by Jimma University. Thus, the authors would like to thank Jimma University for financial support and the Department of Biology, Jimma University, for providing antibacterial activity test.

REFERENCES

Antonio, F. S. *et al*. (2014). Study of the antimicrobial activity of metal complexes and their ligands through bioassays applied to plant extracts. Rev Bras Farmacogn, 24: 309-315.

Edilu, A., Adane, L. and Woyessa, D. (2015). In vitro antibacterial activities of compounds isolated from roots of *Caylusea abyssinica*. Annals of Clinical Microbiology and Antimicrobials, 14:15.

Erin, M. Mc C., Simon, W.J.G., Mark, D.F., Alison, F.K., Waffa, El S. and Declan, P.N. (2008). Antimicrobial activities of pomegranate rind extracts: enhancement by addition of metal salts and vitamin C**.** BMC Complementary and Alternative Medicine, **8**:64.

Graham, L.P. (2013). An Introduction to Medicinal Chemistry 5thed. Great Clarendon Street, Oxford, OX2 6DP, United Kingdom.

Harborne, J.B. (1973). Phytochemical Methods. Chapman and Hall Ltd, London, pp: 49-188.

Mackeen, M.M., Ali, A.M., El-Sharkawy, S.H., Manap, M.Y., Salleh, K.M., Lajis, N.H. and Maffi, L. (1997). Linguistic diversity In: Cultural and Spiritual Values of Biodiversity. Posey D.A. (ed.). London, PP. 1–19.

Muhammad, I.Z., Feroza, H.W., Muhammad, H.S.W., Syed, A.T. and Saad, S. (2012). Antibacterial activities of nicotine and its zinc complex. African Journal of Microbiology Research, 6(24): 5134-5137.

National Committee for Clinical Laboratory standards (2007). Performance standards for antimicrobial susceptibility testing 17th informational supplement.

Neelam, R., Sanjeev, Sh. and Mukta, Sh. (2016). Phytochemical Analysis of Meizotropispellita by FTIR and UV- VIS Spectrophotometer. Indian Journal of Science and Technology,**9**(31).

Prabhjot, S.J., Gagandeep, K. and Loveleen, K. (2015). Synergistic Effect of *Curcuma Longa a*nd *Glycyrrhiza glabra* Extracts With Copper Ions On Food Spoilage Bacteria. Int J Pharm Pharm Sci, 7(10).

Prashanth, M.K., Revanasiddappa, H.D., Rai, K.M.L., Raveesha, K.A. and Jayalakshmi, B. (2013). Antibacterial, anthelmintic and antioxidant activity of *Argyreia elliptica* extracts: Activity enhancement by the addition of metal salts. International Journal of Applied Research in Natural Products, 6(3): 1-10.

Prescott, H. and Klein, J.O. (2002). Microbiology 6th ed. Macgraw Hill Publishers, USA, 808-823.

Safana, A.F. (2013). Study on the interaction of copper (ii) complex of morin and its antimicrobial effect. Int. J. Chem. Sci., 11(3): 1247-1255.

Simon, W.J.G., Mark, D.F., Alison, F.K. and Declan, P.N. (2009). Anti-microbial activities of pomegranate rind extracts: enhancement by cupric sulphate against clinical isolates of *S. aureus*, MRSA and PVL positive CA-MSSA. BMC Complementary and Alternative Medicine, 9: 23.

Subhan, M.A. *et al*. (2014). Synthesis and Characterization of Metal Complexes Containing Curcumin ($C_{21}H_{20}O_6$) and Study of their Anti-microbial Activities and DNA Binding Properties.J. Sci. Res.,6 (1): 97-109.

Subhasish, S., Dharumadurai, D., Sarvanan, C. and Annamalai, P. (2009). Synthesis, Characterization and Antimicrobial Activity of Cobalt Metal Complex against Drug Resistant Bacterial and Fungal Pathogens. Physics, Chemistry and Technology, 7 (1): 73-80.

Tehmina, S., Zahra, Y., Hina, I., Zakir-Ur-R. and Nudrat, F. (2013). Effect of Metal Salts on Antibacterial Activity of *Zingiber Officinale Roscoe* Extract. J. Chem. Soc. Pak., 35(3).

Trease, G.E. and W. C. Evans (1989). Pharmacognosy. 13th ed. Balliere Tindall, London, pp: 176-180.

Yevgen, P., Sule, E., Omer K., H. A., Suheyla, K. and Sıddık, I. (2005). UV/VIS spectral properties of novel natural products from Turkish lichens. International Journal of Photoenergy, 07.

Corresponding author: Gezahegn Faye, Department of Chemistry, College of Natural Sciences, Jimma University, P.O. Box 378, Jimma, Ethiopia
Email: gez.wak1@yahoo.com