International Journal of Researches In Biosciences, Agriculture & Technology | Feb. 2015 Special Issue-1 | ISSN No. (Online) 2347-517X

Synthesis of Piperine Derivatives from Isolated Piperine and their Antimicrobial Activity

N. E. Kathale and P. V.Mandal
Sardar Patel College, Chandrapur - 442402
kathale.niren@gmail.com

Abstract:

In this article, the synthesis of piperine derivatives and their antimicrobial activity on ***Staphylococcus aureus and Escherichia coli*** is studied**.** Piperine derivatives show influential antimicrobial properties in different medium. Synthesis of acylated, sulphonated and hydroxyl derivatives is done by the classical methods. Microbial activity was carried out in sterilized Muller Hinton Agar which was poured into sterilized petriplatesandwas allowed to solidify. Broth was poured on solid agar surface.Sterile micropipette are used to place stock extract of species into each well as per the depth of well with the increasing concentration. After that plates were incubated at 37^0c for 24 hours. Each experiment was performed in triplicate.Activities shown by all the derivatives shows inhibitory action on some microbials are reported.

Keywords: Piperine derivatives, influential antimicrobial properties, adduct formation method

Introduction:

The term alkaloids alkali-like, alk-alkali are basic – nitrogen compounds isolated from plants. “Alkaloids can be defined as naturally occurring organic bases which contain a pyridine ring.” In other way, “Basic nitrogenous plant product mostly optically active and possessing nitrogen heterocycles as their structural units, with a pronounced physiological action are alkaloids.”According to W. Pelletier, alkaloids are defined as a cyclic organic compound containing nitrogen in a negative oxidation state, which is of limited distribution among living organism. Over three thousand alkaloids are known to occur in nature and it is estimated that they are present in only 10-15% of all vascular plants.[4,5] A general method of isolation of alkaloids consistsof extraction of the dried and powdered plant material by refluxing with a suitable solvent. Piperine is an alkaloid, which occurs in pepper, especially unripe black pepper and in the kernel of the ripe fruit. “The piperine content of black pepper being 6 to 11 percent. It is present in relatively smaller amounts in other Piper species e.g. Piper Longum, Piper Lowong”.[7]It was first prepared by Oersted in 1819 and was crystallized as monoclinic crystals having the flavor and taste of black pepper. It is an optically inactive crystalline solid and possesses very weakly basic character. The sharp taste of pepper is however not due to piperine but due to an isomeric compound called **chavicine**. “Piperine is much less toxic than most alkaloids. It acts as local irritant. It is practically insoluble in water but soluble in common organic solvents. It exhibit cis-trans isomerism”.[11,12,13]

Many researchers hadreported, “antidepressant-like effects of piperine (PIP) and its derivative, antiepilepsirine (AES), were investigated in two depressive models: forced swimming test (FST) and tail suspension test (TST)”.[1] To further

explore the mechanisms underlying their antidepressant-like activities, "The brain monoamine levels and monoamine oxidase A and B (MAO-A and MAO-B) activities were also determined".[2,3] The research results for the first time indicated that after two weeks of chronic administration, PIP and AES at doses of 10–20 mg/kg significantly reduced the duration of immobility in both FST and TST, "Antidepressant properties of PIP were supposed to be mediated via the regulation of serotonergic system, whereas the mechanisms of antidepressant action of AES might be due to its dual regulation of both serotonergic and dopaminergic systems". [6,10,14]

The present work is focused on the antimicrobial properties of piperine derivatives. Initially the piperinederivatives are prepared and the antimicrobial activities are studied. Thereafter the various antimicrobial are compared to different in-vitro tissues.

Experimental

Solvent ethyl alcohol AR (99.9%) was procured from ChangshuYangyuanChemicals, China.Diethyl ether 99% AR is obtained from Burgoyne Burbidged and Co. Mumbai, India. Sodium hydroxide pellets 97.5% and Nitric acid- 69-70%was obtained from Thomas BakerChemicalsPvt. Ltd. Mumbai, India. Potassium hydroxide98% was procured from Qualicheme Laboratories. Bezenen95%,was from SD Fine chemicals Limited make.

Isolation of Piperinefrom Pepper

A solution of 50 grams of powdered black pepper in EtOH 250 mlis refluxed in a round bottomed flask fitted with a water condenser for 3 hrs. The resulting solution is filtered andthe filtrate is transferred to 500 ml round bottom flask and the condenser is set for downward distillation. Most of the alcohol is distilled of so that 25-30 ml EtOHremains in the flaskat the end. This concentrated extract is transferred to a porcelain dish, to which 25ml 10% alcoholic KOH solution is addedand then kept at room temperature for 5-10 min. Alcohol is decanted and the insoluble residue is kept overnight in refrigerator. Piperine crystallizes out in form of yellow crystals, m.p. 124-126^{0}c. [15,16]

Sulphonation

1.5g piperine compound is completely saturated with diethyl ether and it is then refluxed with themixture (3ml conc.H_2SO_4 + 2g $HgSO_4$) in the ratio 2:5 H_2O and ethyl alcohol in R.B flask. The reaction mixture is then poured on crushed ice taken in beaker with 2 g KOH with constant stirring.Precipitates of sulphonatedpiperine are obtained after 6-8 hours in the form of dark yellow crystals.

Hydroxylation

2g piperine compound is completely saturated with diethyl ether and it is then reflexed with 1g NaOH and 1g KOH in 10 ml water and 4 ml H_2O_2 for 1 hour in the R.B. flask.The reaction mixture is the cooled overnight in the refrigerator. The hydroxyl derivativeofpiperine crystallizes out in the form of green crystalsare then filtered and dried.

Acylation

Piperine compound is completely saturated with diethyl ether and a mixture of acetic anhydride and $AlCl_3$(Lewis acid) is added to it. This mixture is refluxed for 3 to 5 hours and 10ml distilled water is added with 3ml conc. HCl and heated for few minutes. Then 1ml $H_2SO_4$is added followed by addition of ethyl alcohol in beaker containing crushed ice and add 10% KOH.Then the mixture is kept overnight for cooling in the refrigerator. The product is obtained in the form of gray precipitate.

Determination of Antimicrobial Activity of Piperine Derivatives of Extracts against *Staphylococcus aureus and Escherichia coli*

Solid extracts of **derivatives of Piperine** were subjected to antimicrobial activity studied against Escherichia coli broth culture of unknown isolates from piperine sample and antimicrobial study.

Agar Well Diffusion Method

The sterilize Muller Hinton Agar was poured into sterilized Petri plates and allowed to solidify. After solidification 0.1 ml of broth was poured on solid agar surface by L shaped spreader the stock extract of species was placed into each well with sterile micropipette as per the depth of well with the increasing concentration . The Petri plates were then delicately handled and refrigerated at 4^0c for 2 hours to allow the diffusion of species **Piperine derivatives of extracts**. After that plates were incubated at 37^0c for 24 hours. All the plates were examined for any zone of growth inhibition and diameter of zone of inhibition was measured in millimeter. 0.1 g of only piperine was loded into one of the well as control for comparison.

Result and Discussion:

I] Isolation and enumeration of microorganism

Isolation colonies were obtained after incubation on nutrient agar plates their colonies were studied for its elevation margin colour, pigmentation etc, and the result of colony characteristics are interpreted in table no.1.

From the above observation (Figure 1) most of the colony appeared to be white or yellowish colour.

II) Characterization of microorganism

Isolated colonies were transferred on nutrient agar slants and maintained as a pure culture identification of unknown isolated were carried out by studying morphological culture and biochemical characteristics. Isolates no. 1 is found to be gram negative, rod shape and motile and isolates no 3 was found to be gram positive and non motile. The result of morphological character was interpreted.

III) Determination of antimicrobial activity

The objective of this study was to evaluate the antimicrobial activity of piperine derivatives against various pathogenic bacteria.At the end of the analyses, the derivatives of piperine were found to have an inhibitory effect against all of the test stains. The most susceptible bacteria to cinnamon were S. aureusandE.coli.

Analysis ofH^1-NMR of Hydroxyl derivatives of piperine Data Interpretation

MHz 400.23, CD_3OD δ 0.915(t,2H), 1.36-1.689(quintet,4H),1.967(t,4H), -C**H**-OHδ 3.617(t,1H)protonon the α carbon are deshielded by the electronegative oxygen atom and shifted downfield in the spectra. CH-O**H**no coupling because of the rapid chemical exchange of the –OH in many solution and coupling not usually observed between the –O**H** proton and those –C**H** attached to the α carbon. 4.815(Sp. Singlet 2H),5.946(q,1H),6.585-6.846(multiples,2H),6.884(S,1H), 7.276-7.337(quartet,2H).

Analysis ofH^1- NMR of Acyl derivatives of piperine Data Interpretation

MHz 400.23, CD_3OD δ 0.918(Sp, Singlet, 3H),1.313(t, 2H), 1.674-1.701(quintet, 4H), 1.967(t,4H), 3.8(sp,Singlet, 1H), 3.6(t,1H) 4.811(Sp, Singlet 2H), 5.946(d, 1H), 6.60-6.90(multiples,2H),6.926(S,1H), 7.28-7.342(quartet,2H). The sharp singlet peak δ value 0.90 three proton acyl group (CH_3CO-) is obtained in the H^1-NMR. This signal clearly indicates the introduction of acyl group in piperine molecule is successful.

Table. 1-

Sr.no.	Colony	Growth character
1.	Colony-I	Shape- Regular circular convex, Colour- white, Margin- entire
2.	Colony-II	Shape- Rough circular smooth, Colour- Pale Yellow, Margin- Erose
3.	Colony-III	Shape- Regular circular, convex Colour- Dark Yellow, Margin- Entire

Table. 2- Zone of inhibition of Piperine derivatives by agar well diffusion method.

Piperine derivatives	***E. coli***	***S. aureus***
Pure Piperine	10mm	11mm
Sulphonation	24mm	27mm
Hydroxylation	15mm	16mm
Acylation	16mm	15mm

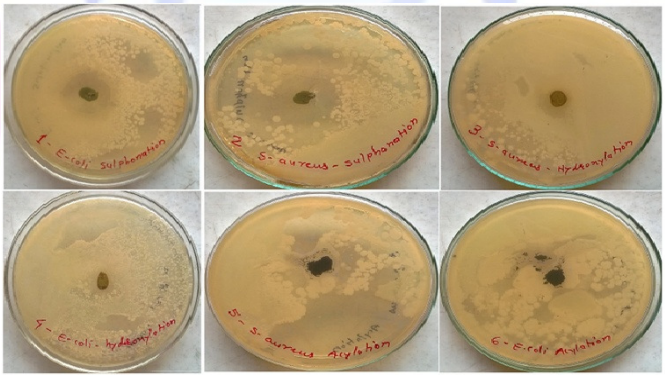

Figure. 1- Anti-microbial activities on Piperine derivatives at 24 hours

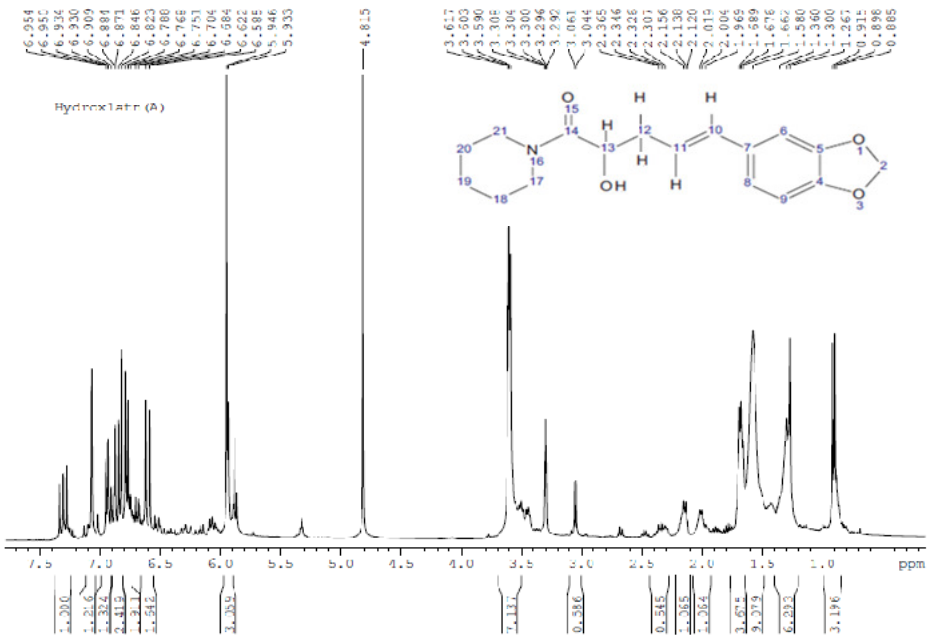

Figure. 2- H^1- NMR of Hydroxyl derivatives of piperine

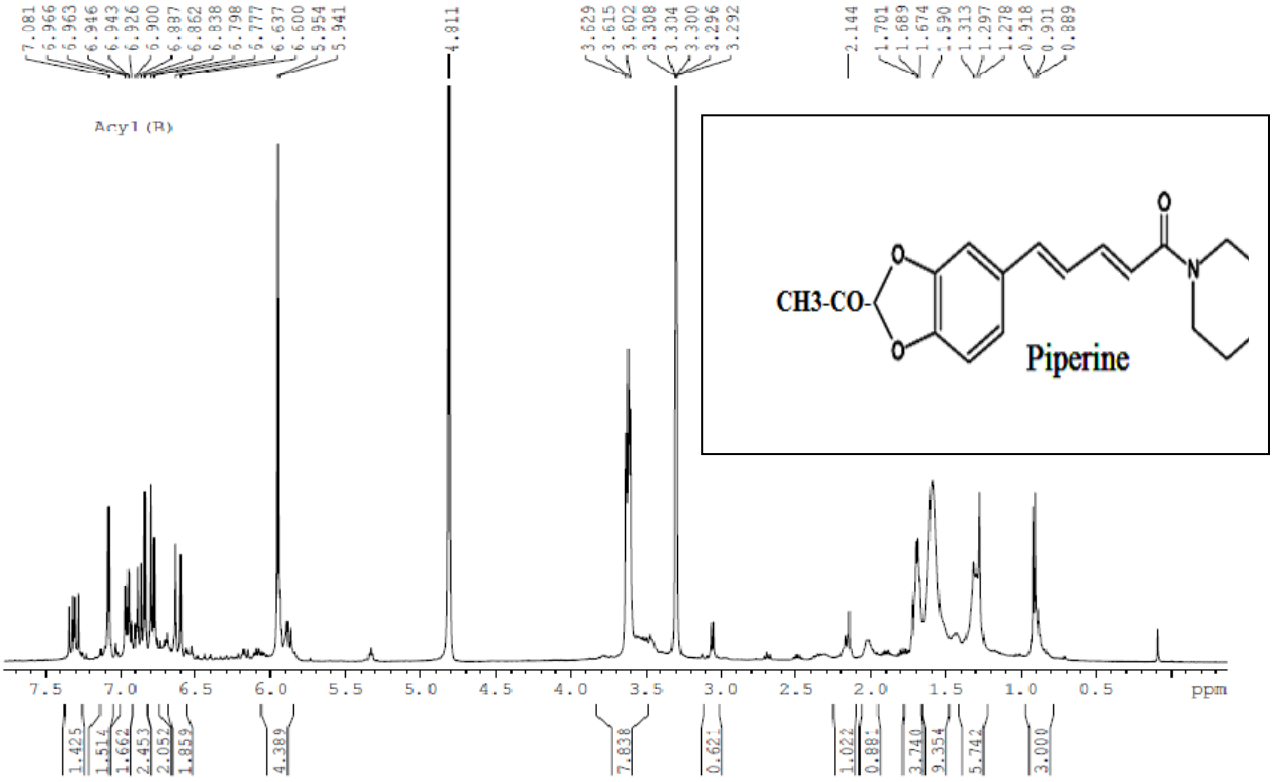

Figure. 3- H^1- NMR of Acyl derivatives of piperine

Conclusion:

The **Piperinederivatives**was taken to study to screen and submit a report on antimicrobial study and also as certain their use in traditional or ethnic medicine.The medicinal Piperine derivatives provides safe and effective remedies for

ailments of both microbial effectiveness of traditional spices herbs against micro-organisms as a result; Piperine derivatives are one of the modern medicines to attain new principles.Its efficiency against Escherichia coli, Staphylococcus aureus by agar well diffusion method. It was found that Piperine derivatives were more effective than aqueous extracts of black pepper.It is observed that the potency of extract is enhanced by the type of solvent used it indicating that there are some active ingredients and chemical constituent i.e. CH_3CO- and -OH which have high antimicrobial effect. The result suggests that the aqueous derivatives of piperine are highly active against both Gram positive and Gram negative bacteria. In conclusion found to have important antimicrobial activity against the test strains. In this regard, the use of derivatives of Piperineand their volatile compounds as natural preservatives in food products; may be an alternative to the use of chemical additives.

References:-

[1] **Aggarwal BB, Kumar A, and Bharti AC.**Anticancer potential of curcumin:preclinical and clinical studies. Anticancer Res. 2003;23, pp.363.

[2] **Aggarwal BB, Sundaram C, Malani N, Ichikawa H. Curcumin:** the Indian solid gold. Adv. Exp. Med. Biol. 2007;595: pp1–75.

[3] **Anand P, Kunnumakkara AB, Newman RA,** Bioavailability of curcumin: problems and promises. Mol.Pharm. 2007;4: pp807–18.

[4] **D.T. Wong, F.P. Bymaster.**Prog. Drug Res., 58, 169 (2002).

[5] **E. Dailly, F. Chenu, C.E. Renard.**Fund.Clin.Pharmacol., 18, 601 (2004).

[6] **Goel A., Kunnumakkara A.B., Aggarwal B.B.,** "Curecumin": from kitchen to clinic" Biochem. Pharmacol. 2008;75: pp787–809.

[7] **H.A. Dhaenen, A. Bossuyt.** Biol. Psychiatry, 35, 128 (1994).

[8] **I. Malagie, D.J. David, P. Jolliet, R. Hen, M. Bourin, and A.M. Gardier** Eur. J. Pharmacol., 443, 99 (2002).

[9] **J.A. Gordon, R. Hen.**Annu.Rev. Neurosci., 27, 193 (2004).

[10] **Jagetia G.C., Aggarwal B.B.,** "Spicing up of the immune system by curcumin", J. Clin. Immunol. 2007;27: pp 19–35.

[11] **K. Wei, W. Li, and K. Koike.** J. Nat. Prod., 67, 1005 (2004).

[12] **L.D. Kong, C.H. Cheng, and R.X. Tan.** J. Ethnopharmacol., 91, 351 (2004).

[13] **McGonagle, and S. Zhao.** "Arch. Gen. Psychiatry", 51, 8 (1994),(20).

[14] **W.E. Muller.Pharmacol.**Res., 47, 101 (2003).

[15] **P. Blier, and F.V. Abbott.** J. Psychiatry Neurosci., 26, 37 (2001).

[16] **Shishodia S., Sethi G. and Aggarwal B.B.** "Curcumin: getting back to the roots", Ann. NY Acad. Sci. 2005;1056: pp. 206–17.

[17] **Wang, F. Xu and R.R.**Gainetdinov,"Biol. Psychiatry", 46, 1124 (1999).