ISOLATION AND SYNTHETIC MODIFICATION OF CUBEBIN FROM *PIPER CUBEBA* SEEDS

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THRIKKAKARA)

SUBMITTED BY:-

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VI Semester
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June 2020

CERTIFICATE

This is to certify that the project entitled "ISOLATION AND SYNTHETIC MODIFICATION OF CUBEBIN FROM *PIPER CUBEBA* SEEDS" was carried out by ANN MARY JOSEPH under my supervision and guidance. The work presented here has not been reported for my other degree or diploma.

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DECLARATION

I hereby declare that this project entitled "**Isolation and synthetic modification of cubebin from *Piper cubeba* seeds**" is a record of bonafide work carried out during my course of study under the guidance of Dr. Sajin Francis K, Department of chemistry, Bharata Mata College Thrikkakara.

Place: Thrikkakara

Date: 08/06/2020

Ann Mary Joseph

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Ann Mary Joseph

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INTRODUCTION

Human beings have depended on nature for their simple requirements as being the sources for medicines, shelters, food stuffs, fragrances, clothing, flavours, fertilizers and means of transportation throughout the ages. For the large proportions of world's population medicinal plants continue to show a dominant role in the healthcare system and this is mainly true in developing countries, where herbal medicine has continuous history of long use.The development and recognition of medicinal and financial aids of these plants are on rise in both industrialized and developing nations [1].

The foundations of typical traditional systems of medicine for thousands of years that have been in existence have formed from plants. The plants remain to offer mankind with new medicines. Some of the beneficial properties ascribed to plants have recognized to be flawed and medicinal plant treatment is based on the experimental findings of hundreds to thousands of years. The earliest reports carved on clay tablets in cuneiform date from about 2600 BC are from Mesopotamia; among the materials that were used were oils of *Commiphora*species (Myrrh), *Cedrus*species (Cedar), *Glycyrrhiza glabra* (Licorice), *Papaver somniferum*(Poppy juice) and *Cupressus sempervirens* (Cypress) are still used today for the cure of diseases extending from colds and coughs to inflammation and parasitic infections [2].

The traditional medicine practice is widespread in China, India, Japan, Pakistan, Sri Lanka and Thailand. About 40% of the total medicinal consumption is attributed to traditional tribal medicines alone by China. In Thailand, herbal medicines make use of legumes encountered in the *Caesalpiniaceae*, the *Fabaceae*, and the *Mimosaceae*. It is estimated that in mid-90s, more than US$2.5 billion have resulted from the sales of herbal medicines. The herbal medicinal preparations are more in demand than mainstream pharmaceutical products in Japan.

Even today, plants are not only indispensable in health care, but form the best hope of source for safe future medicines [3]. In spite of the fact that now we have at our command a number of modern drugs, it is still genuinely urgent to discover and develop new therapeutic agents. It has been estimated that the acceptable therapy is available only for one third of the known human ailments. Therefore, the fight against diseases must be carried on relentlessly. Traditional plant medicines still enjoy significant position

in the modern-day drug industries due to the minor side effects as well as the synergistic action of the combination of compounds.

Most of the important drugs of the past 50 years, which have revolutionized modern medicinal practice, have been isolated/derivatized from plants. These chemical ingredients exhibit therapeutic properties of plant and animal drugs. The WHO endorses and promotes the addition of herbal drugs in national health care programs because they are easily accessible at a price within the reach of a common man and are time tested and thus considered to be much safer than the modern synthetic drugs [4]. Thus, the research of pharmacologically/ biologically active agents obtained by screening natural sources such as plant extracts had led to the detection of many pharmaceutically valuable drugs that play a key role in the treatment of human diseases [5]. The phytochemical-pharmacological research work has recently yielded effective solutions to certain diseases which synthetic drug industry has failed to afford. The most important among them are the research work on *Artimisiaannua, Cathranthus roseus, Taxus spp., Lantana camara*and *Baccopa spp*. etc. Such plants were earlier considered as poisonous or useless, but now have been found to contain molecules of high drug values and are considered as medicinal herbs of great significance.

PIPER SPECIES

In these modern times, the concept of a return to the “roots” of medicine is starting to become more and more popular. Scientific progress has provided new approaches for the analysis of different folk herbs that are used in various cultures [6,7]. The pharmacological properties of plants used as food, medicine or for spiritual purposes during the centuries have been confirmed through new approaches to their analyses [8-10]. The heritage of using some plants in traditional medicine is continuously being corroborated in terms of their effects through scientific inquiry [11,12]. One of the widely distributed plant genera in pantropical regions is the genus *Piper*. *Piper* plants are also known under the common name” pepper”. The presence of oil cells in the structures of almost all *Piper* species places them in the group of aromatic plants [13]. Besides their well-known uses as culinary spices, the secondary metabolites isolated from *Piper* plants show wide ranging human health effects.

One of the most extensively studied compounds isolated from *Piper* plants is piperlongumine, also known as piplartine. Piplartine is an amide alkaloid found in several

Piper species (Piperaceae). It has been shown that piplartine has potential anticancer properties [14,15]. Piplartine also shows benefits in the treatment of the parasitic infection schistosomiasis, caused by helminth flatworms of the genus *Schistosoma* [16]. Compounds from *Piper tuberculatum*fruits show antiplasmodial and antileishmanial activities [17]. All these activities of *Piper* plants on neglected tropical diseases are very important for pantropical regions, which are the natural habitats of these plants. Piperlongumine shows anti-inflammatory effects in the central nervous system (CNS). In relationwith inflammation-related brain diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease, one of the potential approaches in prevention and treatment of these diseases is normalization of microglia activity. The anti-neuroinflammatory effects of piperlongumine are characterized as inhibition of the production of nitric oxide (NO) and prostaglandin E2 (PGE2) induced by lipopolysaccharide (LPS), also reducing the expression of inducible nitric oxide synthase and cyclooxygenase-2 as well as proinflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin-6 (IL-6), and also by suppressing the nuclear factor kappa B (NF-κB) signaling pathway [18].

Antimicrobial activity of *Piper* plants has been shown in the treatment of chronic periodontitis [19], as well as in the treatment of gastric pathogen *Helicobacter pylori* [20] and decreased *H. pylori* toxin entry to gastric epithelial cells [21]. In addition to the abovementioned pharmacological activities of *Piper* plants, different investigations have also indicated that these plants are active as anti-diabetic, anti-ulcer, diuretic, and local anesthetic agents [22]. Most of the information about the various biological activities of *Piper* plants has been derived from in vitro studies, while in vivo and toxicology studies are still somewhat limited. However, it can be noticed that these plants have multi-targeting potential, and their underlying mechanisms of action are waiting to be explored [23].

Piper nigrum L.

Narayanan [24] recognizes around 135 compounds in these EOs, belonging to the monoterpenoid, sesquiterpenoid, aliphatic, aromatic and other chemical groups. He states that generally speaking the EOs are composed by 70–80% of monoterpene hydrocarbons (mainly α-pinene up to 13%, β-pinene up to 40%, limonene up to 32%), 20–30% sesquiterpene hydrocarbons (mainly β-caryophyllene up to 22%) and less than 4% oxygenated constituents [24]. A recent paper on different types of Chinese *P. nigrum*

(black, white and green) confirms these ranges with 39.74–

64.67% monoterpene hydrocarbons, 1.85–3.44% monoterpenoids, 20.87–43.89% sesquiterpene hydrocarbons and 2.21–10.81% sesquiterpenoids [25].

As with many of the EOs of the *Piper* genus, the chemical composition is however extremely varied, and Narayanan [24] considers taxonomical differences (varieties), geography, maturity of the raw material, and differences in distilling parameters and analytical techniques as the principal causes of this variety. Major compound classes recognized by Narayanan [24] are the following:

(1) *Monoterpene hydrocarbons*: camphene, δ-3-carene, *p*-cymene, limonene, myrcene, (*Z*)-β-ocimene, α-phellandrene, β-phellandrene, α- and β-pinenes, sabinene, α- and γ-terpinenes, terpinolene and α-thujene.

(2) *Oxygenated monoterpenoids*: borneol, camphor, carvacrol, *cis*-carveol, *trans*-carveol, carvone, carvotanacetone, 1,8-cineole, cryptone, *p*-cymene-8-ol, *p*-cymene-8-methyl ether, dihydrocarveol, dihydrocarvone, linalool, *cis*-*p*-mentha-2,8-dien-1-ol, *p*-mentha-3,8-dien-1-ol, *p*-mentha-1(7),8-dien-1-ol, 1 (7)-*p*-menthadien-4-ol, *p*-mentha-1,8-dien-5-ol, *p*-mentha-1,8-dien-4-ol, *cis*-*p*-menth-2-en-1-ol, myrtenal, myrtenol, methyl carvacrol, *trans*-pinocarveol, pinocamphone, *cis*-sabinene hydrate, *trans*-sabinene hydrate, terpinen-4-ol, 1-terpinen-5-ol, α-terpeneol, phellandral, piperitone, citronellal, nerol, geraniol, isopinocamphone, methyl citronellate, methyl geranate, α-terpenyl acetate, terpenolene epoxide and *trans*-limonene epoxide.

(3) *Sesquiterpene hydrocarbons*: *cis*-α-bergamotene, *trans*-α-bergamotene, β-bisabolene, β-carophyllene, α- and β-cadinenes, calamenene, α-copaene, α- and β-cubebenes, *ar*-curcumene, β- and δ-elemene, β-farnesene, α-guaiene, α-humulene, isocaryophyllene, γ-muurolene, α-santalene, α- and β-selinenes, ledene, sesquisabinene and zingiberene.

(4) *Sesquiterpenoids*: 5,10(15)-cadinadien-4-ol, caryophylla-4(12),8(13)-dien-5β-ol, β-caryophyllene alcohol, caryophyllene ketone, caryophyllene oxide, epoxy-dihydrocaryophyllene, (*Z*)-nerolidol, cubenol, *epi*-cubenol, viridiflorol, α- and β-bisabolols, cubebol, elemol and eudesmol.

(5) *Miscellaneous compounds*: eugenol, methyl eugenol, myristicin, safrole, benzaldehyde, (*E*)-anethole, piperonal, *m*-methylacetophenone, *p*-methylacetophenone, butyrophenone, benzoic acid, phenylacetic acid, cinnamic acid and piperonic acid.

Piper longum l.

A very recent review by Lawrence [26], which it can be seen that the EOs seem to be characterized by non-terpenoid compounds such as pentadecane and heptadecane isomers, and by sesquiterpene hydrocarbons such as germacrene D, β-caryophyllene, and β-selinene. In only one case the characterizing compound is a phenylpropanoid, eugenol.

Piper cubeba l

Piper cubeba L., is a flowering vine commonly known astailed or java pepper belongs to family Piperaceae, genusPiper which is a folkloric plant and has been cultivated inmany countries including India for its fruit and essential oil [27]. In India, Charaka and Sushrutatextsincludedcubeba in various remedies and in traditional Chinese medicine it is used for its alleged warming property.Arab physicians of the Middle Ages, the cubeba was usedunder the name of Kababa, when preparing the water of albutm [28]. The genus Piper is represented withmore than 1000 commercially and medicinally interestingspecies [29]. Several species of this genus werefound to have antiinflammatory, antinociceptive, cytotoxic,antimicrobial, antiprotozoal and antiproliferative activities.The fruits are berry and are used to treat gonorrhea, dysen--tery, syphilis, abdominal pain, asthma [30].

Fig 1: *Piper cubeba* plant and seeds

PHYTOCHEMISTRY

Thirteen different lignans including furanofuran lignans such as cubebin, hinokinin, yatein and isoyatein were appeared in the dried fruit of *Piper cubeba* [31]. About 15% of a volatile oil was obtained by distilling cubebs with water [32].

The physicochemical analysis reported about 4.53% w/w moisture content and higher amount of extractive value 18.71% w/w) in ethanol. The higher nutritive value; 353.95 Cal per 100 g of fruit powder was reported. The reports showed presence of phosphorous 19.52, iron 5.73 and zinc 0.27 ppm in the fruits. The qualitative phytochemical screening of ethanol extract revealed the presence of a wide range of phytoconstituents like carbohydrates, proteins,glycosides, saponins, diterpenes, phenols, flavonoids etc., and all other secondary metabolites [33].

OBJECTIVES OF THE PRESENT STUDY

Piper cubeba is one of the important medicinal plant used in traditional system of medicine. Cubebin, is one of the major phytochemicalspresents in the *P. cubeba*. This compound has wide varieties of medicinal applications. Another pharmaceutically important compound present in this plant is hinokinin, which is minor compound in this plant. Isolation of cubebin and its synthetic modification in to hinokin is described in this project. So the title of this project is **"ISOLATON AND SYNTHETIC MODIFICATION OF CUBEBIN FROM *PIPER CUBEBA* SEEDS".**

MATERIALS AND METHODS

Extraction

Piper cubeba seeds were purchased from local market in Ernakulam. About 100 g of the seeds were dried and coarsely powdered and soaked in chloroform (500 ml) for two days. The solvent is filtered and evaporated solvent under reduced pressure in a rotary evaporator. The extract obtained was 4 g.

Isolation of chemical constituents

The chloroform extract obtained was subjected to column chromatography on silica gel using hexane- ethyl acetate as solvent yielded 93 fractions with 50 ml each. These fractions pooled together in to 13 fractions according to the similarities in TLC. The fraction pool 4 (103 mg) contain a major UV active spot which is submitted to column chromatography on silica gel using 10% Ethyl acetate-hexane as eluent which yielded the

compound 1 (43 mg) as viscous liquid. The fraction pool 5 (1.5g) contain a major UV active spot which is subjected to column chromatography on silica gel using 10% Ethyl acetate-hexane as eluent which yielded the compound 2 as colourless solid (1.3g) . Compound 1 and 2 were characterized using various spectroscopic techniques.

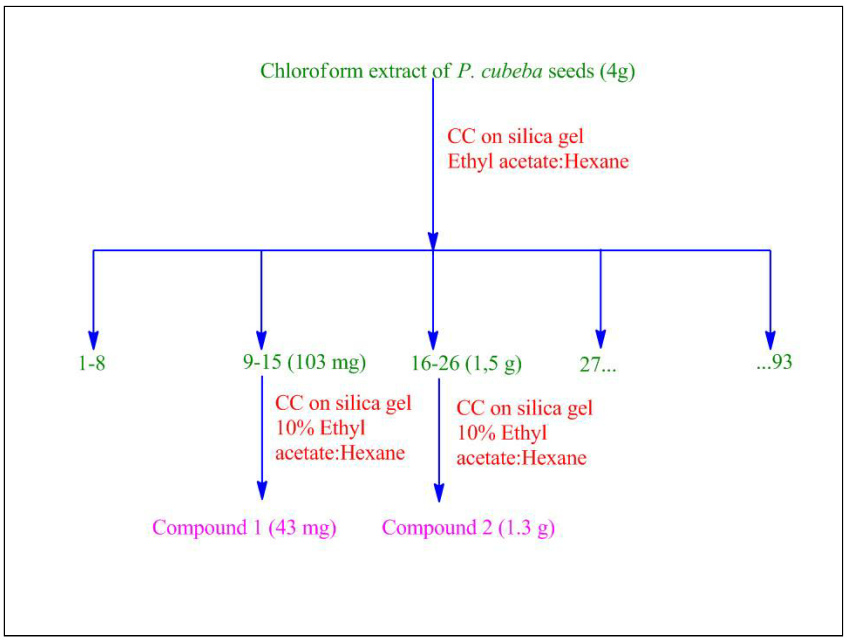
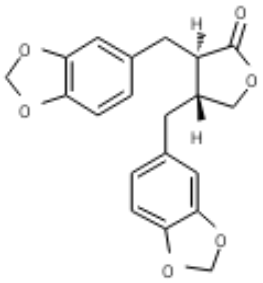


Fig 2: Schematic representation of isolation of compounds

Characterization of compounds

Molecular formula of the compound 1 is $C_{20}H_{18}O_6$ obtained from elemental analysis. Its molecular ion peak is *m/z* 354 (M^+). IR spectrum showed absorption at 1773 cm^{-1} indicated the presence of a lactone ring. ^{1}HNMR showed a singlet at δ 5.93 integrating for 4 protons indicated the presence of two methylenedioxy group. ^{13}C NMR showed a peak at δ 178.4 indicated the presence of latone ring. The compound was identified as hinokinin by comparison with the literature [34]. Structure of the compound 1 is shown below.



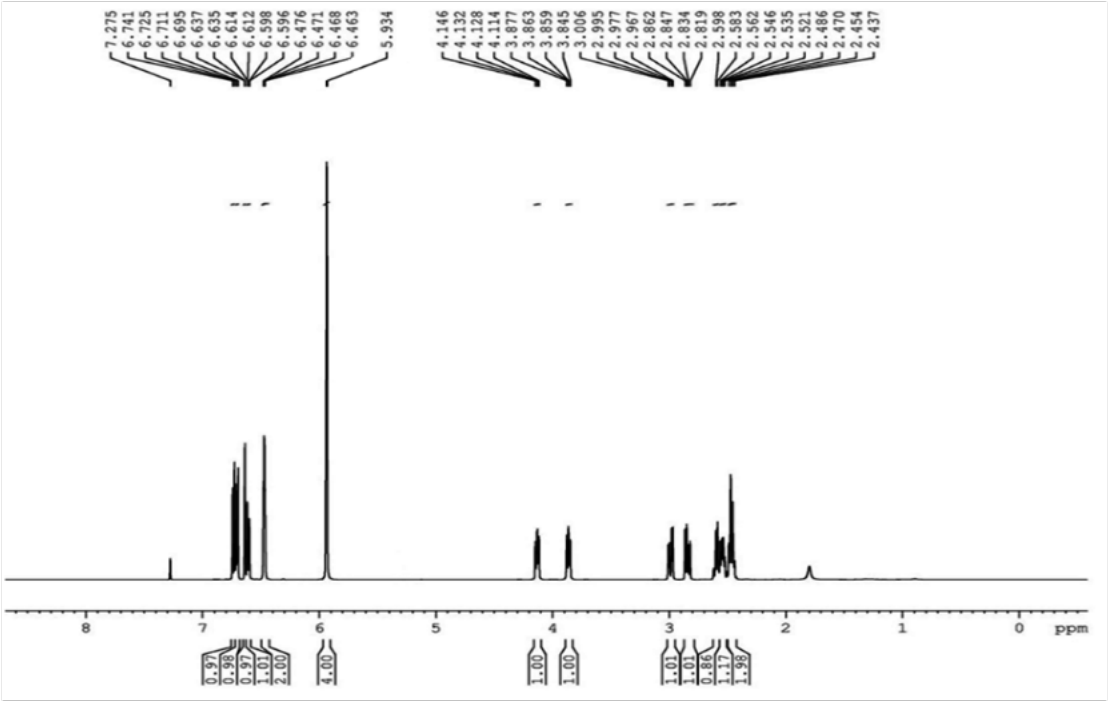


Fig 3: ^{1}H NMR spectrum of hinokinin

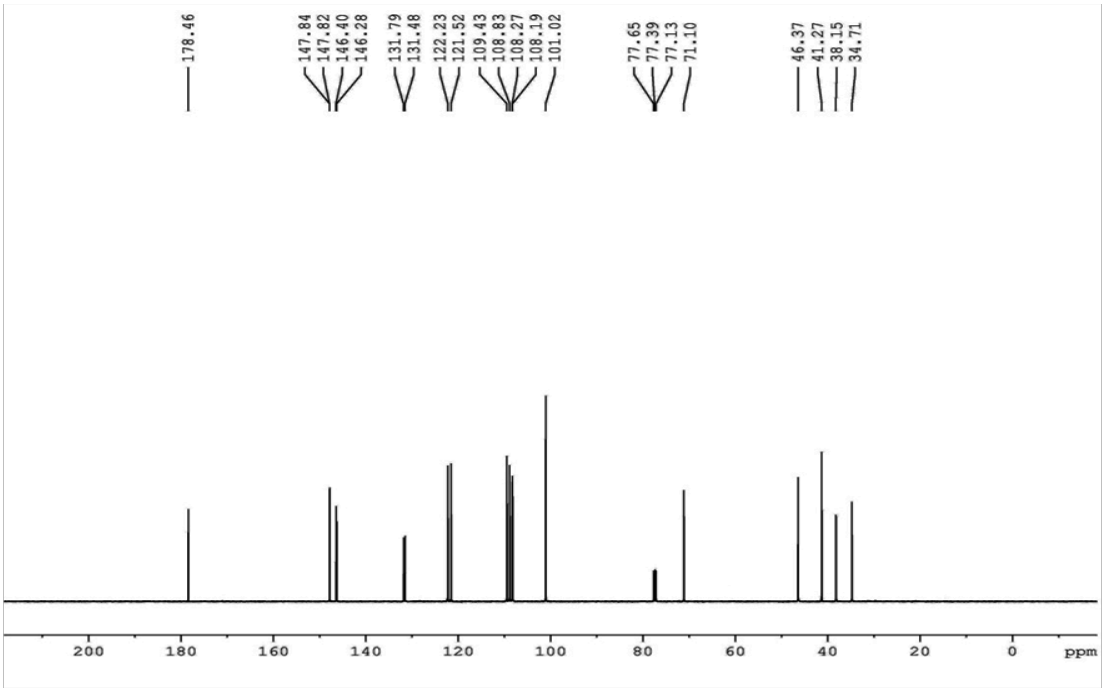
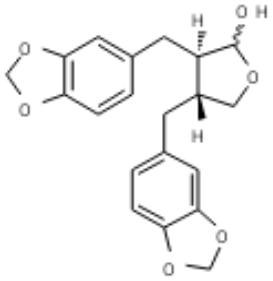


Fig 4: ^{13}C NMR spectrum of hinokinin

Molecular formula of the compound 2 is $C_{20}H_{20}O_6$ obtained from elemental analysis. Its molecular ion peak is *m/z* 356 (M^+). IR spectrum showed absorption at 3427 cm^{-1}

indicated the presence of a hydroxyl group. ^{1}HNMR showed a singlet at δ 5.92 integrating for 4 protons indicated the presence of two methylenedioxy group. The compound was identified as Cubebin by comparison with the literature [35]. Structure of the compound 2 is shown below.



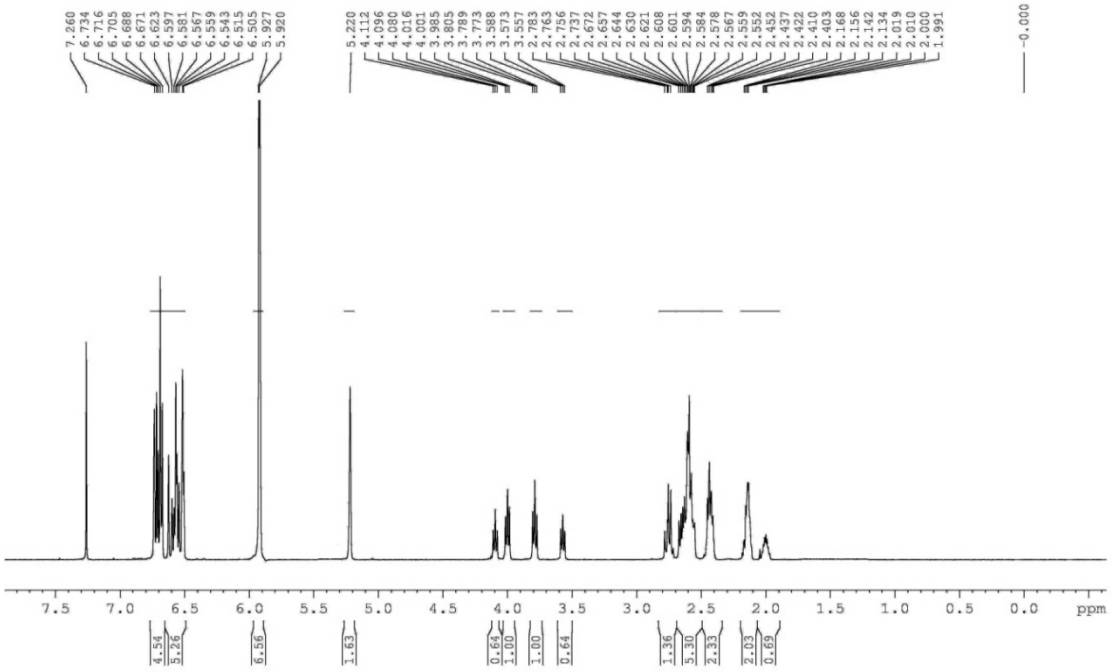


Fig 5: ^{1}H NMR spectrum of cubebin

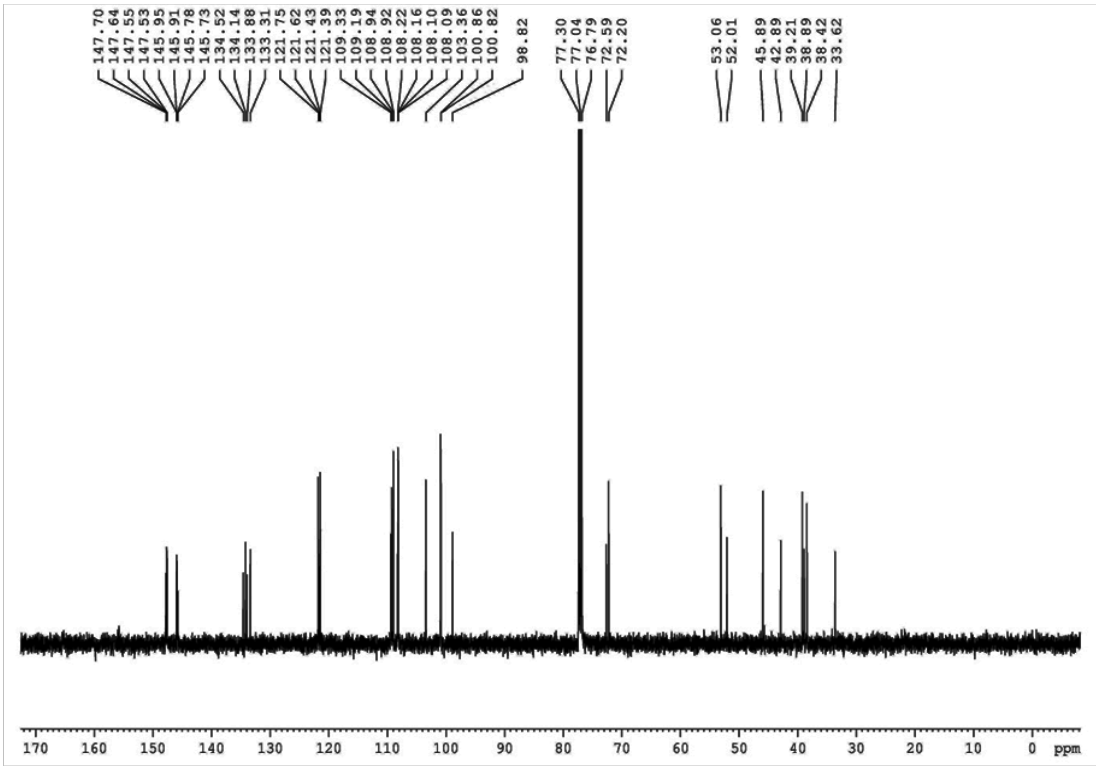


Fig 6: NMR spectrum of cubebin

Conversion of Cubebin to Hinokinin

Cubebin (100 mg) is dissolve in dichloromethane. A stirred solution of cubebin in DCM added pyridinium chlorochromate (0.01 mmoles) for 12 hours. The conversion has been monitored by checking the TLC. After the conversion the solvent is removed under vacuum. The crude reaction mixture is purified using column chromatography [36]. The hinokinin obtained was 60 mg.

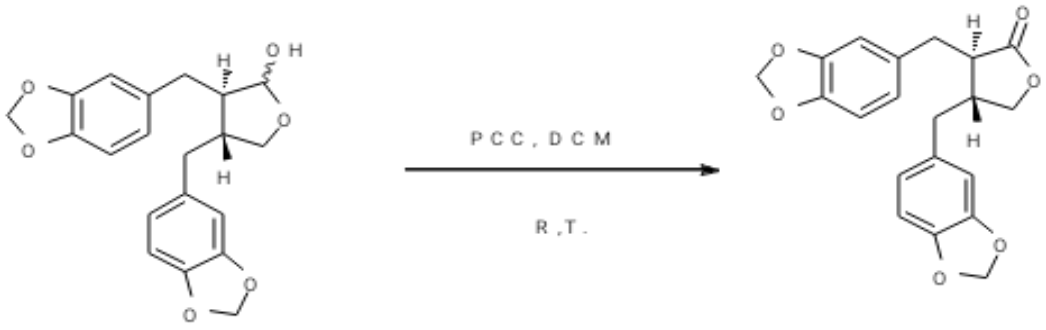


Fig 7: Scheme for the conversion of cubebin to hinokinin

CONCLUSION

Medicinal plants have greater importance in our daily life. One f the important medicinal plant is *Piper cubeba*. It is used in Ayurvedic formulations in India. The major phytochemical present in this plant is cubebin. This compound exhibit therapeutic properties. So in this project we have carried out the isolation of cubebin from *P. cubeba* seed extract. Hinokinin is another minor compound present in this plant. We have successfully converted cubebin into hinokin in better yield.

REFERENCES

[1] WHO, Regulatory situation of herbal medicines. A worldwide review. Geneva, Switzerland.**1998**, 1-5.

[2]Fakim, A.G. "Medicinal plants: Traditions of yesterday and drugs of tomorrow". *Molecularaspects of medicine*, **2006**, 27, 1-93.

[3] Hamburger, M.; Hostettmann, K. "Bioactivity in plants: the link between phytochemistry and medicine". *Phytochemistry*, **1991**, *30*, 3864-3874.

[4] Singh, P.; Singh, C. L. "Chemical investigations of *Clerodendraonfragrans*". *Journal Indian Chemical Society*, **1981**, *58*, 626-627.

[5] Rastogi, P. R., Meharotra, B. N. In Compendium of Indian Medicinal Plants. Vol. I, 339; a) (1993) III: 194. PID, CSIR, New Delhi, India, **1990**.

[6] Salehi, B.; Hernández-Á lvarez, A.J.; Contreras, M.D.M.; Martorell, M.; Ramírez-Alarcón, K.; Melgar-Lalanne, G.; Matthews, K.R.; Sharifi-Rad, M.; Setzer, W.N.; Nadeem, M. "Potential phytopharmacy and food applications of Capsicum spp.: A comprehensive review". *Nat. Prod. Commun.***2018**, *13*, 1543–1556.

[7] Sharifi-Rad, M.; Ozcelik, B.; Altın, G.; Daşkaya-Dikmen, C.; Martorell, M.; RamírezAlarcón, K.; Alarcón-Zapata, P.; Morais-Braga, M.F.B.; Carneiro, J.N.P.; Alves Borges Leal, A.L. "Plants-from farm to food applications and phytopharmacotherapy". *Trends Food Sci. Technol.***2018**, *80*, 242– 263.

[8] Mishra, A.P.; Sharifi-Rad, M.; Shariati, M.A.; Mabkhot, Y.N.; Al-Showiman, S.S.; Rauf, A.; Salehi, B.; Župunski, M.; Sharifi-Rad, M.; Gusain, P. "Bioactive compounds and healthbenefits of edible Rumex Rumex species—A review". *Cell. Mol. Biol. (Noisy-le-grand)***2018**,*64*, 27–34.

[9] Sharifi-Rad, M.; Fokou, P.V.T.; Sharopov, F.; Martorell, M.; Ademiluyi, A.O.; Rajkovic, J.; Salehi, B.; Martins, N.; Iriti, M.; Sharifi-Rad, J. "Antiulcer agents: From plant extracts to phytochemicals in healing promotion". *Molecules* **2018**, **23**, 1751.

[10]Abdolshahi, A.; Naybandi-Atashi, S.; Heydari-Majd, M.; Salehi, B.; Kobarfard, F.; Ayatollahi, S.A.; Ata, A.; A.; Tabanelli, G.; Sharifi-Rad, M.; Montanari, C. "Antibacterialactivity of some Lamiaceae species against *Staphylococcus aureus* in yoghurt-based drink (Doogh)". *Cell. Mol. Biol. (Noisy-le-grand)* **2018**, *64*, 71–77.

[11] Mishra, A.P.; Saklani, S.; Salehi, B.; Parcha, V.; Sharifi-Rad, M.; Milella, L.; Iriti, M.; Sharifi-Rad, J.; Srivastava, M. "*Satyrium nepalense*, a high-altitude medicinal orchid of Indian Himalayan region: Chemical profile and biological activities of tuber extracts". *Cell.Mol. Biol.* **2018**, *64*, 35–43.

[12] Salehi, B.; Sharopov, F.; Martorell, M.; Rajkovic, J.; Ademiluyi, A.O.; Sharifi-Rad, M.; Fokou, P.V.T.; Martins, N.; Iriti, M.; Sharifi-Rad, J. "Phytochemicals in *Helicobacter pylori* infections: What are we doing now?" *Int. J. Mol. Sci.* **2018**, *19*, 2361.

[13] Dyer, L.A.; Palmer, A. "Piper A Model Genus for Studies of Phytochemistry, Ecology, and Evolution; Springer: Heidelberg, Germany, **2012**; ISBN 978-0306484988.

[14] Raja Mazlan, R.N.A.; Rukayadi, Y.; Maulidiani, M.; Ismail, I.S. "Solvent extraction and identification of active anticariogenic metabolites in *Piper cubeba L.* through ^{1}H-NMR-basedmetabolomics approach". *Molecules* **2018**, *23*, 1730.

[15]Bezerra, D.P.; Ferreira, P.M.P.; Machado, C.M.L.; de Aquino, N.C.; Silveira, E.R.; Chammas, R.; Pessoa, C. do Ó . "Antitumour efficacy of *Piper tuberculatum* and piplartine based on the hollow fiber assay". *Planta Med.* **2015**, *81*, 15–19.

[16] Campelo, Y.; Ombredane, A.; Vasconcelos, A.G.; Albuquerque, L.; Moreira, D.C.; Plácido, A.; Rocha, J.; Fokoue, H.H.; Yamaguchi, L.; Mafud, A. "Structure–Activity relationship of piplartine and synthetic analogues against *Schistosoma mansoni* and cytotoxicity to mammalian cells". *Int. J. Mol. Sci.* **2018**, *19*, 1802.

[17]De Souza Oliveira, F.A.; Passarini, G.M.; de Medeiros, D.S.S.; de Azevedo Santos, A.P.; Fialho, S.N.; de Jesus Gouveia, A.; Latorre, M.; Freitag, E.M.; de Maria de Medeiros, P.S.; Teles, C.B.G. "Antiplasmodial and antileishmanial activities of compounds from *Pipe tuberculatum* Jacq fruits". *Rev. Soc. Bras. Med. Trop.* **2018**, *51*, 382–386.

[18] Kim, N.; Do, J.; Bae, J.S.; Jin, H.K.; Kim, J.H.; Inn, K.S.; Oh, M.S.; Lee, J.K. "*Piper longumine* inhibits neuro inflammation via regulating NF-kappaBsignaling pathways in lipopolysaccharide-stimulated BV2

microglia cells". *J. Pharmacol. Sci.***2018**, *137*, 195–201.

[19]Gamboa, F.; Muñoz, C.C.; Numpaque, G.; Sequeda-Castañeda, L.G.; Gutierrez, S.J.; Tellez, N. "Antimicrobial activity of *Piper marginatum* Jacq and Ilex guayusa Loes on microorganisms associated
with periodontal disease". *Int. J. Microbiol*. **2018**, *41*, 73-83.

[20] Tharmalingam, N.; Kim, S.-H.; Park, M.; Woo, H.; Kim, H.; Yang, J.; Rhee, K.-J.; Kim, J."Inhibitory effect of piperine on Helicobacter pylori growth and adhesion to gastric adenocarcinoma cells". *Infect.*
Agent Cancer 2014, *9*, 43.

[21]Tharmalingam, N.; Park, M.; Lee, M.H.; Woo, H.J.; Kim, H.W.; Yang, J.Y.; Rhee, K.J.; Kim, J.B. "Piperine treatment suppresses *Helicobacter pylori* toxin entry in to gastric epithelium and minimizes β-
catenin mediated oncogenesis and IL-8 secretion in vitro". *Am. J. Transl. Res.* **2016**, *8*, 885–898.

[22] Durant-Archibold, A.A.; Santana, A.I.; Gupta, M.P. "Ethnomedical uses and pharmacological activities
of most prevalent species of genus Piper in Panama: A review". *J. Ethnopharmacol.***2018**, *217*, 63–82.

[23] Choudhary, N.; Singh, V. "A census of *P. longum*'s phytochemicals and their network pharmacological
evaluation for identifying novel drug-like molecules against various diseases, with a special focus on
neurological disorders". *PLoSONE*, 2018, *13*, e0191006.

[24] Narayanan, C.S. "Chemistry of Black Pepper. In Black Pepper—Piper nigrum; Ravindran, P.N., Ed.;
Harwood: Amsterdam, The Netherlands, **2005**, 147–166.
[25] Liu, H.; Zheng, J.; Liu, P.; Zeng, F. "Pulverizing processes affect the chemical quality and thermal
property of black, white, and green pepper (*Piper nigrum* L.)". *J. Food Sci. Technol.***2018**, *55*, 2130–2142.

[26] Lawrence, B.M. "Progress in essential oils: Long pepper oil". *Perfum. Flavor*. **2015**, *40*, 42–44.

[27] Dodson, C. D.; Dyer, L. A.; Searcy, J.; Wright, Z.; Letourneau, D. K. "Cenocladamide, a diydropyridone
alkaloid from *Piper cenocladum*". *Phytochem*. **2000**, *53*, 51-54.

[28]Mabberley, D. J. The plant book: A portable dictionary of the higher plants; utilizing Kubitzki's The
families and genera of vascular plants (1990), Cronquist's anintegrated system of classification of flowering plants (1981) and current botanical literature arranged largely on the principles of ed. 1-6 (1896/97-1931) of Willis's A dictionary of the flowering plants and ferns. Cambridge [u.a.]: Cambridge
Univ. Press. 1997.

[29]Khare, C. P. Indian herbal remedies: rational western therapy, Ayurvedic and other traditional usage,
Botany, Springer, **2004**.

[30] Eisai, P. T. Medicinal Herb Index in Indonesia. Dian Rakyat, Jakarta, 2nd edition, **1995**. 21.

[31]Elfahmi, R. K.; Batterman, S. “Lignan profile of *Piper cubeba*, an Indonesian medicinalplant”. *Biochem*
Syst Ecol. 2007, *35*, 397–402
[32] Lawless.; Julia. The illustrated encyclopedia of essential oils:the complete guide to the use of oils in
aromatherapy and herbalism. Element Books, 1995.

[33]Muchandi, A. A.; Dhawale, S. C. “Pharmacognostic evaluation and free radical scavengingactivity of
ethanolic extract of *Piper cubeba* fruits”. *Asian J Phytomed Clin Res*. 2017, *5*, 42-52.

[34] Koul, S. K.; Taneja, S. C.; Dhar, K. L.; Atal, C. K. Lignans of *Piper clusil, Phytochemistry,* 1983, *22*, 999-1000,

[35]Bharathi, R. P.; Newand, B. M. Lignans from *Piper cubeba*, *Phytochemistry*, 1985, *24*, 329- 331.

[36] Vanessa, A. de.; Rosangela, da. S.; Ana, C. P.; Vanessa de, A. R.; Juliana, S.; Marisa, M.; Gustavo H. B.
de Souza.; Ademar, A.; Filho, S.; Marcella, D.; Paulo, M. D.; Jairo, K. B.; Se´rgio, A.; Ma´rcio, L. A.
“Trypanocidal activity of (-)-cubebin derivatives against free amastigote forms of *Trypanosoma cruzi*”.
Bioorganic & Medicinal Chemistry Letters. 2005*15*, 303–307.