Part I

METAL-AMINE REDUCTION ON CARBOCYCLIC COMPOUNDS AND BIOCIDAL ACTIVITY OF THE DERIVED DERIVATIVES.

CHAPTER I

This chapter is divided into two sections, Section A and Section B

SECTION A

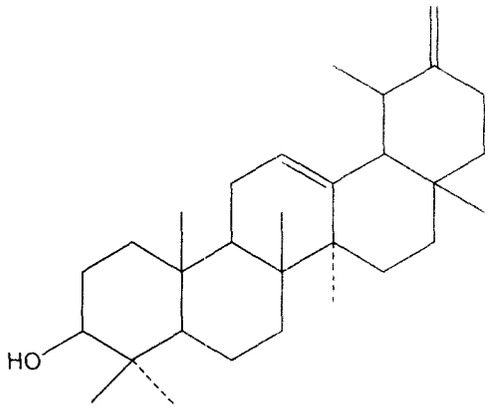
A SHORT REVIEW ON THE METAL-AMINE REDUCTION ON CARBOCYCLIC COMPOUNDS

Alkali metals in presence of bases are known as reducing agent for the reduction of organic compounds since early years. The well known Birch reduction is the first example where these types of reagents are used. The reaction of unsaturated organic compounds with alkali metals and alcohols in liquid ammonia is called Birch [1-5] reduction. This method was first used for reduction of aromatic compounds by Wooster [6]. However, its general recognition and wide application was achieved only after a series of investigations by Birch [7]. According to Birch's method [8], the alkali metal (sodium or potassium) was added to a well stirred mixture of an alcohol (taken in stoichiometric amount with respect to the metal), liquid ammonia and the substance to be reduced.

Regel and co-workers [9] reported first time a new metal-amine reduction system, lithium in ethylenediamine. They [9] reported that lithium in presence of ethylenediamine reduced aromatic rings to mono-olefins and to cyclo parafins; reduced phenols; cleaved ethers; reduced ketones to alcohols and reduced acetylene and bond terminal and internal olefins to alkanes. Regel et al. [9] believed that it would be possible to reduce any carbon-carbon double or triple bond with lithium in ethylenediamine. It was also shown [10] that this isomerisation was catalyzed by the amide-

$$H_2N\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}\bar{N}HLi^+$$

Corey et al. [11] used lithium in presence of ethylenediamine for selective reduction of olefinic double bond on triterpenoids. They obtained α-amyrin 1a from ursa-12: 20(30)-dien-3β-ol, 1.



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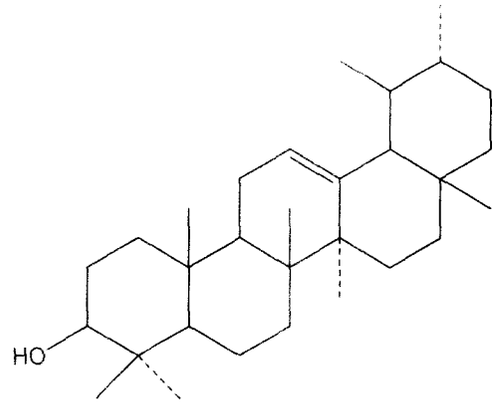


1a

Regel et al. [12] also studied the isomerisation of olefins and dehydrogenation of cyclic dienes with lithium in presence of ethylenediamine. They [12] suggested the following mechanism for the isomerisation and dehydrogenation.

Mechanism for isomerisation of olefins

$$R\text{-}CH_2\text{-}CH{=}CH_2 + (H_2N\text{-}CH_2\text{-}CH_2\text{-}\bar{N}H)$$

$$\Downarrow$$

$$(R\text{-}\bar{C}H\text{-}CH{=}CH_2) + H_2N\text{-}CH_2\text{-}CH_2\text{-}NH_2$$

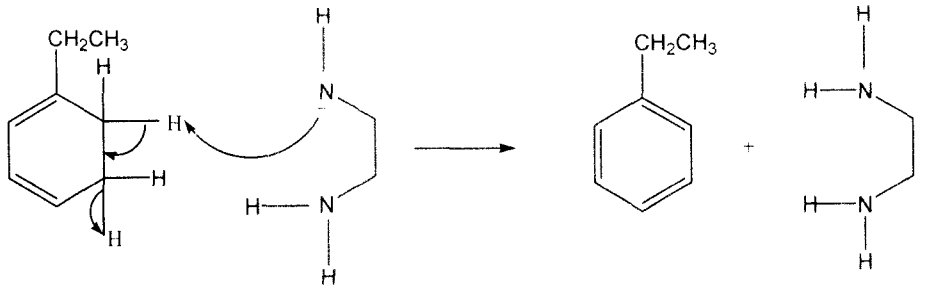
$$\Updownarrow$$

$$R\text{-}CH{=}CH\text{-}\bar{C}H_2 + H_2N\text{-}CH_2\text{-}CH_2\text{-}NH_2$$

$$\Downarrow$$

$$R\text{-}CH{=}CH\text{-}CH_3 + (H_2N\text{-}CH_2\text{-}CH_2\text{-}\bar{N}H)$$

Mechanism for dehydrogenation of cyclic diene



Tyagi et al. [13] also studied the behavior of cyclopropane and cyclobutane rings on many triterpenoids towards the reagent (lithium-ethylenediamine).

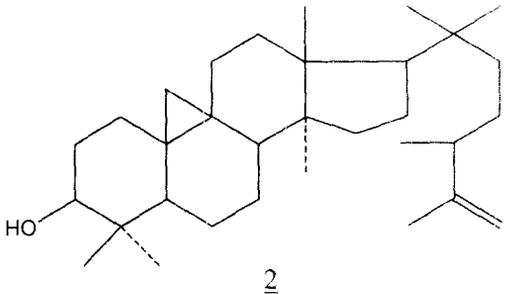
Smith et al. [14] dehydrogenated primary and secondary alcohols carbonyl compounds in presence of lithium metal in ethylenediamine.

Tyagi et al. [15] further reported that N-lithioethylenediamine is useful for partial aromatization of cadinenic terpenoids, for conversion of selenenic compounds to heteroannular diene and for smooth isomerisation of fatty acids.

Oscer et al. [16] reported that lithiated-diamines could be used to metalate a variety of weakly acidic compounds. N-lithioethylenediamine reacted smoothly with a variety of amines like aniline, diphenyl amine and hydrocarbons like phenyl acetylene, fluorene and indene at room temperature.

$$RH + NH_2CH_2CH_2NHLi \rightleftharpoons RLi \,.\, NH_2CH_2CH_2NH_2$$

Narula et al. [17] reported that cyclolaudenol 2 on exposure to N-lithioethylenediamine at 120-125^{0}C gave the isopropylidene isomer 2a in 92% yield.



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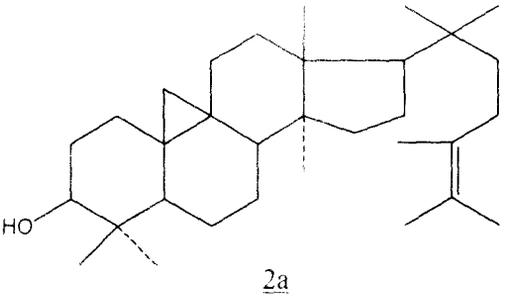
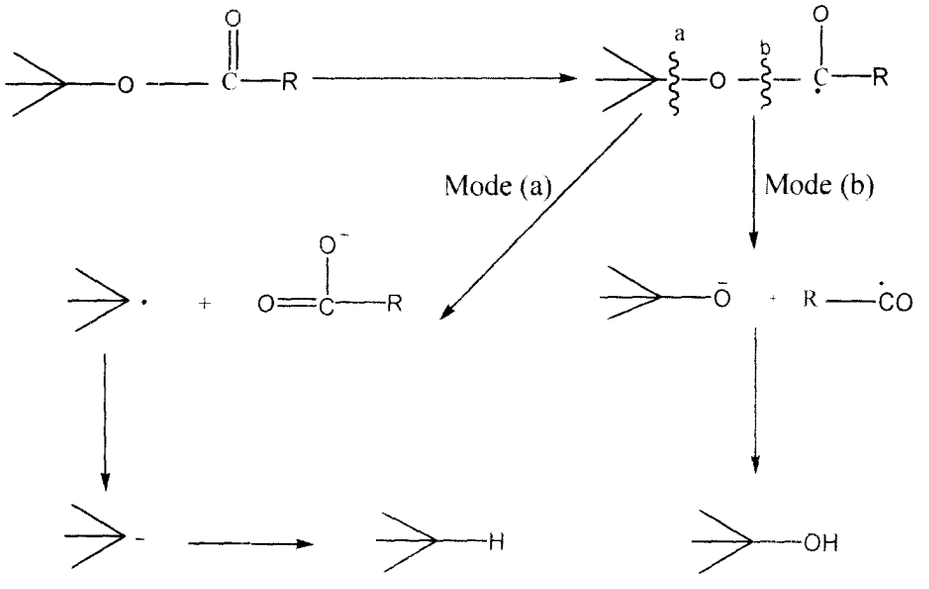


2a

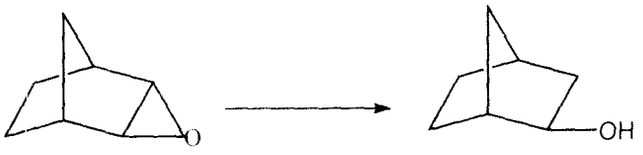
Boar et al. [18] established that sterically hindered alcohols were conveniently and efficiently converted into the corresponding alkanes by metal-amine reduction of the derived esters with carboxylic acids and suggested the following mechanism (Scheme-1).

Scheme-1



Brown et al. [19] reported that many hindered and unstable bicyclic epoxides were reduced very rapidly without rearrangement by lithium metal in presence of

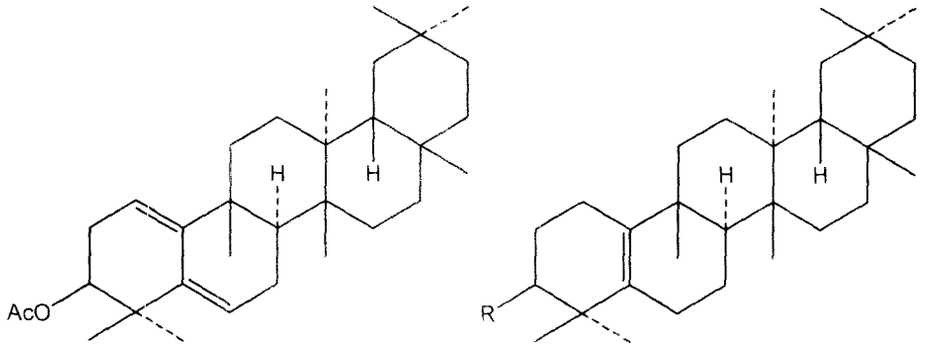
ethylenediamine. In this way, norbonene oxide 3 was rapidly reduced to essentially pure 2-norbornanol, 3a, in 87% yield.



3 3a

Benkeser et al. [20] recently have shown that lithium in ethylamine is a powerful and selective reducing agent towards aromatic rings. The aromatic systems are rapidly reduced to monoolefins which are themselves far more slowly reduced. Implicit in the tentative mechanism proposed is the facile reduction of nonaromatic conjugated systems, specifically 1,3-dienes. It is interesting to use the Benkeser reagent with norbornadiene to provide a different system for evaluating conjugation effects in that compound. Since isolated double bonds are reduced with difficulty, it was expected that if the compound were reduced readily, nortricyclene would be formed by homoconjugative 1,5-addition.

Sengupta et al. [21] studied the reduction of lithium in ethylenediamine on triterpenoid heteroannular 1,3-dienes and observed that gluto-1(10),5-dienyl-3β-acetate



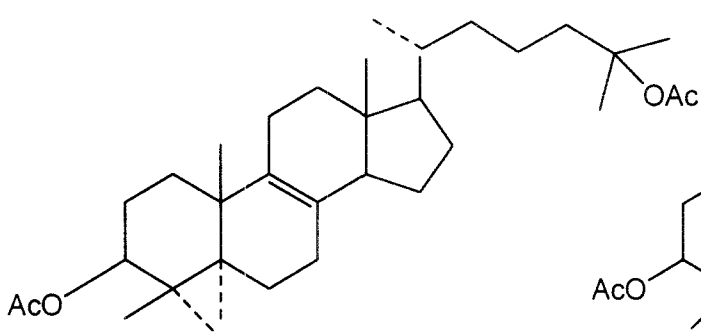
4 5a. R = H

5b. R = OH

4 gave a mixture of deoxygenated product, glut-5(10)-ene, 5a and hydrolysed product, glut-5(10)-en-3β-ol, 5b.

They [22] also studied the same reaction on hindered triterpenoid esters and observed that methyl esters of oleanolic acid, crategolic acid, prodocarpic acid and ursolic acid were hydrolysed to their corresponding acids under the reaction condition.

Barrett et al. [23] studied the reduction of steroidal systems containing two ester groups in sterically different environments. Compounds 6 and 7 when treated with lithium in ethylenediamine the products 8, 9 and 10 were obtained respectively.



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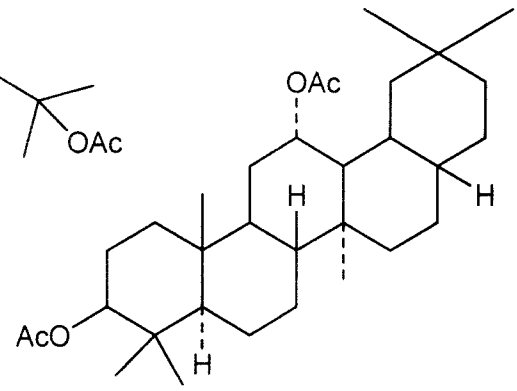

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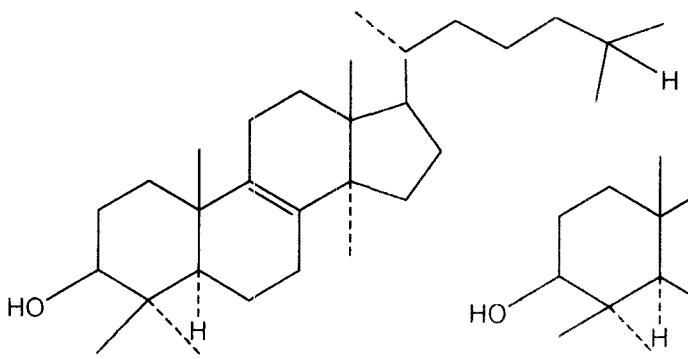

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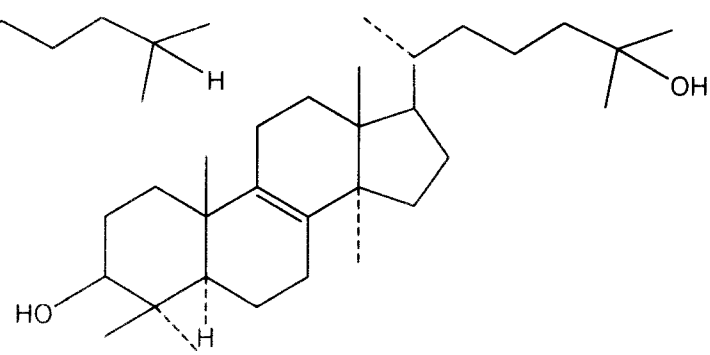

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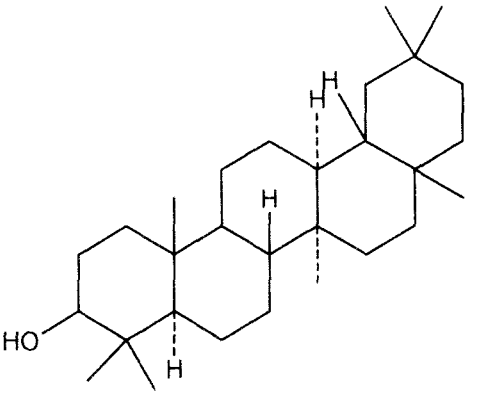

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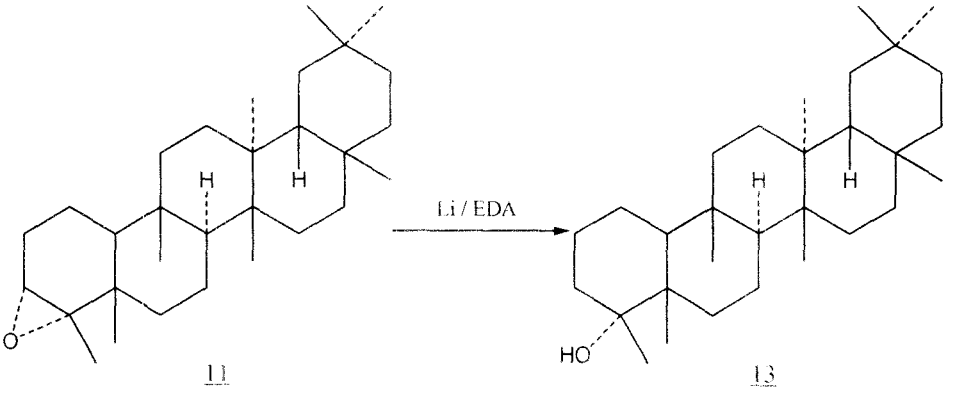
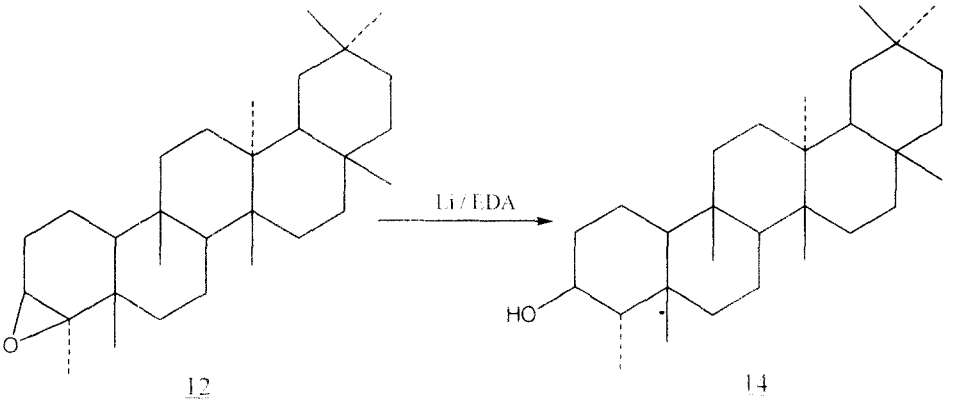
Sengupta et al. [24] used lithium in ethylenediamine for ring opening of triterpenoid epoxides. They [24] reported that the reaction of lithium in ethylenediamine with 3α,4α-epoxyfriedelan [25] 11 and 3β,4β-epoxyfriedelan [26] 12 afforded 4α-hydroxyfriedelan 13 and 4-epifriedelan-3β-ol 14 via epoxide ring opening respectively.

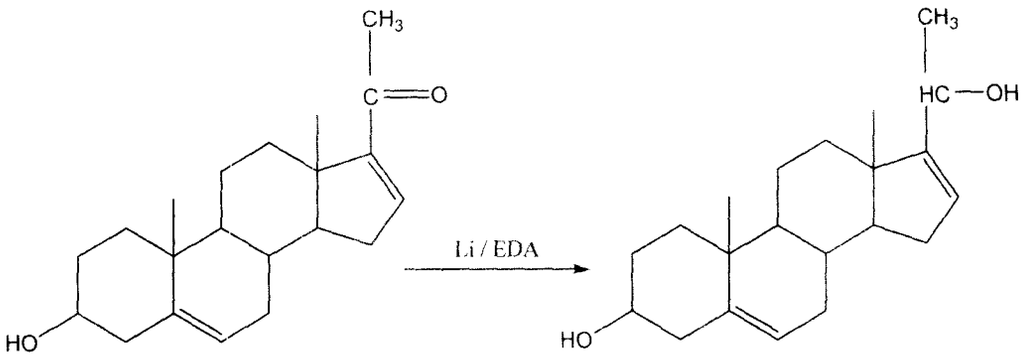


11 13



12 14

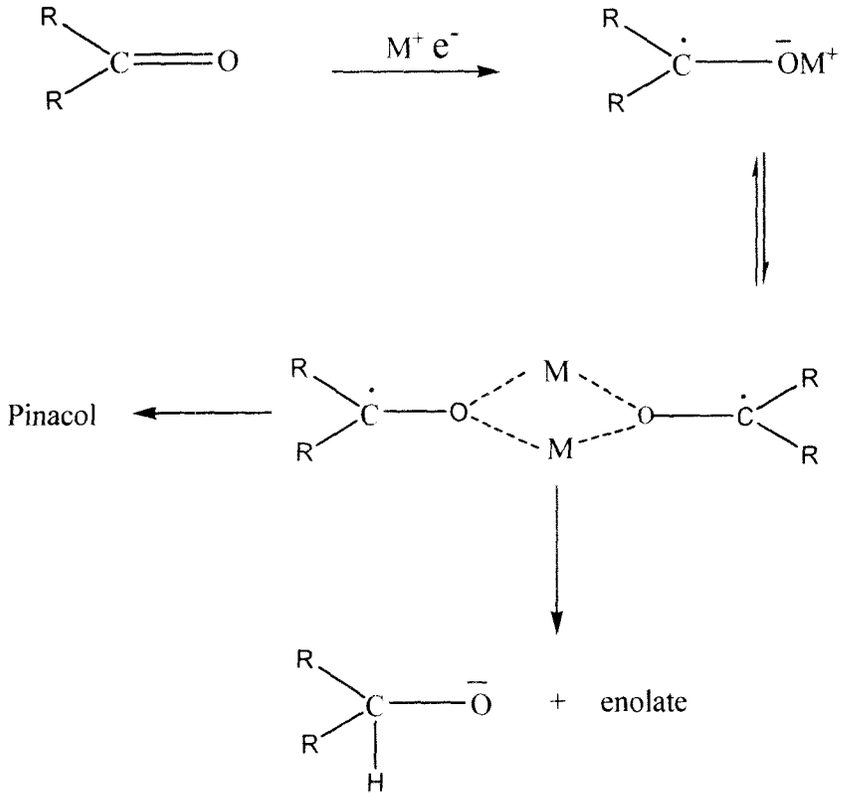
They [27] also carried out the same reaction for the complete stereoselective reduction of an α,β-unsaturated ketone. They observed that $\Delta^{16\text{-}20}$ keto function in $\Delta^{5,16}$-pregnadiene-3β-ol-20-one 15 was reduced stereoselectively to Δ^{5}-pregnene-3β, 20R-diol 16 in excellent yield on treatment with lithium in boiling ethylenediamine.



15 16

Rautenstrauch et al. [28] found that a non-enolizable ketone, 2,2,6,6-tetramethylcyclohexanone reacted with one mol equivalent of lithium in THF to give lithium ketyl aggregates which were stable at low temperature and were not reduced further with excess of lithium. These results, combined with detailed studies on the reduction of (+) camphor-3-d_2 demonstrated that in the absence of proton donars the reduction of enolizable saturated ketones by dissolving metals proceeded via hydrogen transfer, presumably within a ketyl dimer [29]. Based on these and large body of other data, a unified mechanism for the reduction of saturated ketones by dissolving metals both in the presence and absence of added proton donars was given by Huffman [30] (Scheme-II).

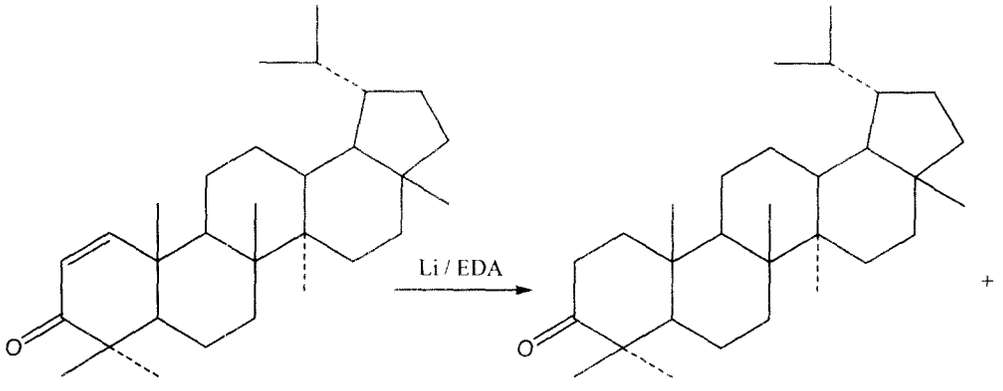
Scheme-II



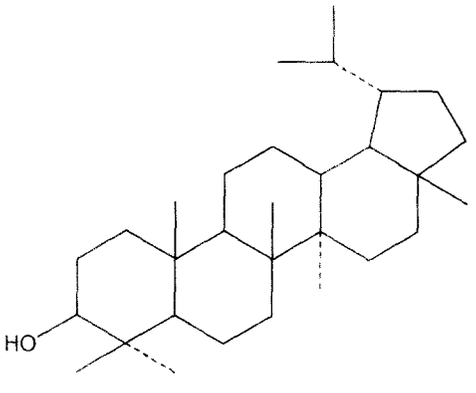
Huffman et al. [31] carried out reduction of (+) camphor using Li, Na and K in THF and compared the results with those in ammonia. The results were identical in both the systems. From these results they [31] concluded that these reactions followed the same path in ammonia and THF which they had proposed previously. Huffman et al. [31] carried out the reaction of fenetone (0.8 mL) with excess (3 mole) Li/THF at 25^{0}C and the data of these reactions were consistent with a persistent radical anion which existed in the presence of excess of lithium and not consistent with a dianion intermediate proposed by Pradhan [32]. Pradhan et al. [33] reported the wide applicability of lithium-ethylenediamine in the reduction of ketones and aldehydes to alcohols, isopropenyl groups to isopropyl groups and esters of hindered acids to carboxylic acids of a series of triterpenoids containing one or more of these functional groups. They [34] were also successful in reducing α,β-unsaturated carbonyl

functionality of triterpenoids and steroids with this reagent. By investigating the reaction on less sterically hindered ketone 17 and sterically hindefed ketone 17a, they suggested that lithium-ethylenediamine could be a potential reducing agent for the reduction of α,β-unsaturated ketones (Scheme-III) especially when such a system is a part of steroids and triterpenoids.

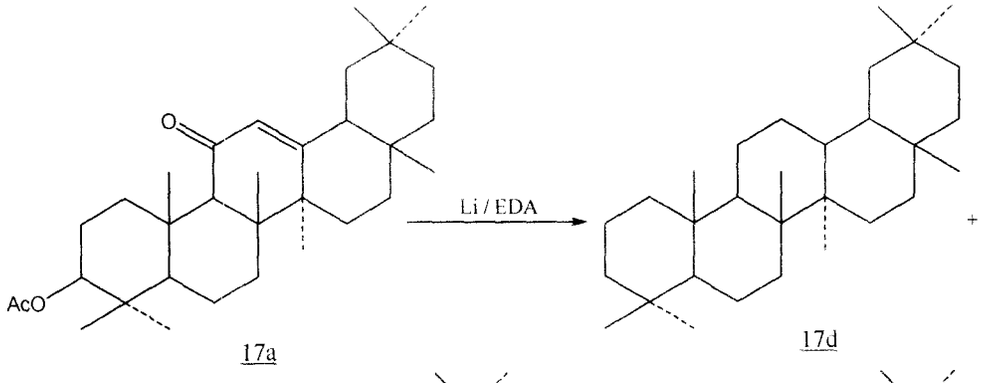
Scheme-III



17 17b

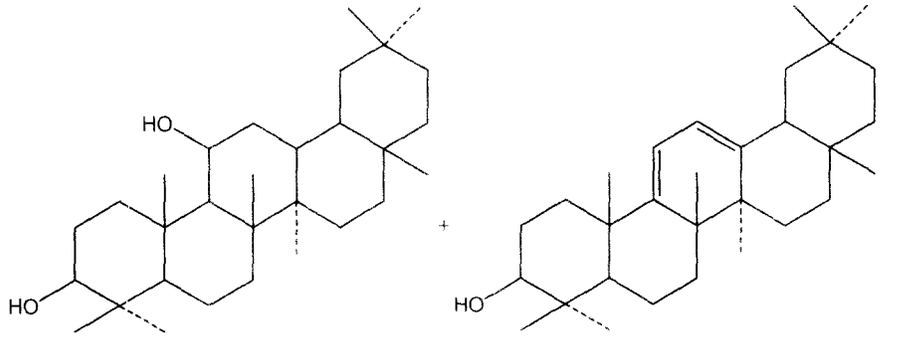


17c



17a

17d

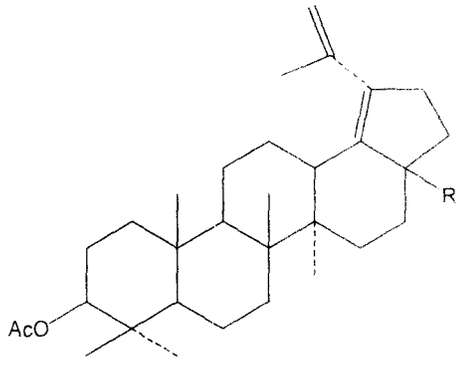


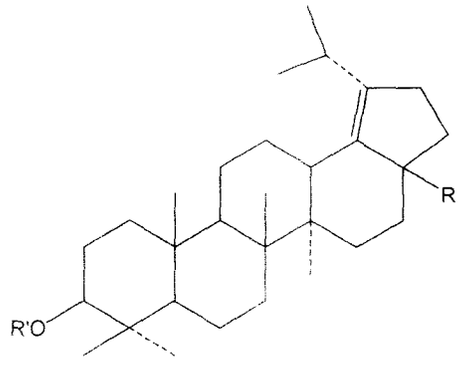
17e

17f

While working on the structure elucidation of the triterpenes odolactone and acetylodolactone, Pradhan et al. [35] reported the opening of lactone ring for the first time by lithium-ethylenediamine.

Pradhan et al. [36] investigated the reaction of lithium-ethylenediamine on





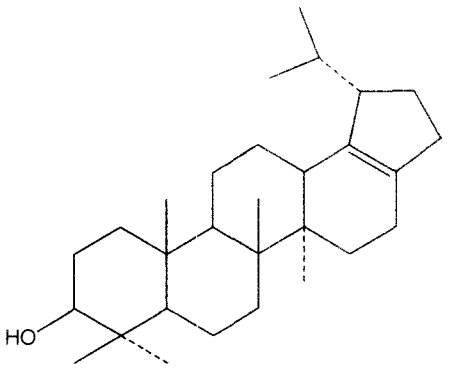
18a. R = CH_3

18b. R = COOMe

19. R = CH_3

19a. R = CH_2OH, R' = H

19b. R = COOH, R' = H



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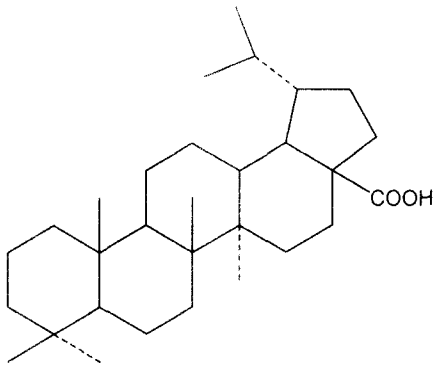

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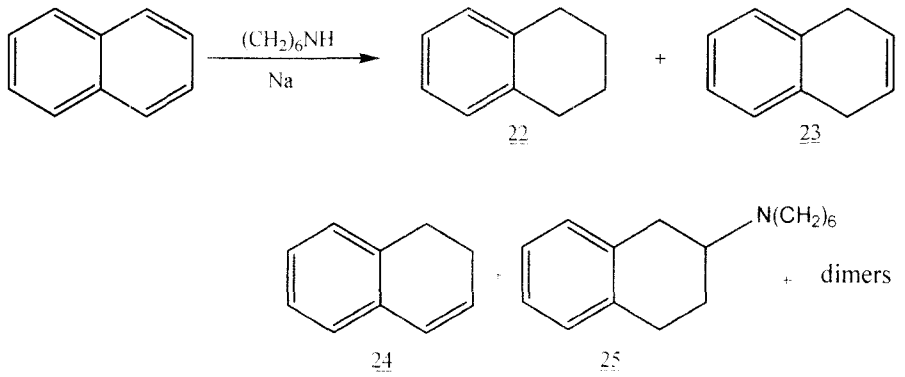
triterpenoid conjugated ketones and reported the isolation of 19 from 18a, 20, 19a, 21 and 19b from 18b respectively.

Eisenbraun et al. [37] demonstrated that amines and sodium may be used in the reduction of naphthalene and alkylated napthalenes to dihydronaphthalenes and tetrahydronaphthalenes. However, in some cases, the low yields of reduced hydrocarbons pointed to the formation of side reaction products. These were identified as hydrocarbon dimers and secondary or tertiary amines, of which N-(1,2,3,4-tetrahydro-2-naphtyl) hexamethylenimine 25 is typical. The hydrocarbon reduction products accompanying 25 are shown in scheme IV. In this case of naphthalene, 22 is the dominant volatile hydrocarbon product and with certain amines, the presence of 23 can be detected in the early stages of the reaction. The tertiary amine 25 results from amination of 24.

Scheme IV



22 23 24 25

From the above it is observed that although various applications of metal-amine reduction have been performed on a host of compounds still there exist ample scope to extend the reaction on various functionalities. Studies on the effectiveness of other alkali metals in similar kind of application are also not yet explored. Thus it was felt necessary to include the above study on some selected carbocyclic compounds in the present investigation

SECTION B

A SHORT REVIEW ON THE BIOCIDAL ACTIVITY OF THE CHEMICAL CONSTITUENTS ISOLATED FROM THE PLANT EXTRACTS

Since the present investigation includes screening of the biocidal activity of the functionally transformed triterpenoids in comparison to their parent compounds along with 2,3-disustituted pyrazine derivatives, it was felt necessary to present a short review of the biocidal activity of the triterpenoids isolated from plants or of the crude plant extracts as a whole or of the prepared related derivatives in brief.

Throughout history, mankind has always been interested in naturally occurring compounds from prebiotic, microbial, plants and animals sources. Various extracts of different parts of plants have been widely used in folk medicines and perfumes as well as in food flavor and preservatives and are more commonly utilized in chronic diseases like cancer, diabetes and asthma.

The ancient Egyptians have described several useful preparations such as opium and castor oil. They also used "rotten bread" for treating infections which resembles our use of antibiotics from moulds and fungi.

The Chinese are considered as leaders in using natural products for healing. The oldest compilation of Chinese herbs is Shen Nung Pen Ts'ao, which lists 385 materials. 5267 medicinal herbs were used in China in 1967. One of the most famous herbs among them is the ginseng root, *Panax ginseng* is used for health maintenance and for the treatment of various diseases. Another popular folk drug is the extract of the Ginkgo tree, *Ginkgo biloba* which can improve memory and mental alertness.

During the 17^{th} century, the Jesuite brought with them from South America the bark of the China tree for the treatment of malaria. In 1820, Pelletia and Caventou isolated from the China tree the active compound, quinine. American Indians used the powerful hallucinogen, mescaline for a long period. The Indian hemp plant, *Cannabis sativa*, has been used since 3000 BC, and it is used as marijuana or hashish.

At the onset of the present study it was considered to review the reports presented by the earlier workers regarding the biocidal activity of various plant extracts

tested on different organisms, especially on them selected for the present investigation. The observation of the previous workers in concord with the present line of investigation is being presented, in a selective manner, in the following paragraphs.

Mansouri [38] found new antibacterial agents from ethanolic extracts of ten plants. The agents were effective against *Staphylococcus aureus*. Several samples (489 samples) of *S. aureus* were isolated from healthy carriers (nose and throat) or clinical samples. Out of 489 isolates 98.6% were sensitive to trimethoprim-sulfamethoxazole. The extracted compounds from the plants were screened for antibacterial activity. *Myrtus communis* (L.) showed the greatest activity, inhibiting the growth of 99% of the isolates. *Glycyrrhiza glabra* (L.), *Eucalyptus globolus* (Labill) and *Menta vividis* (L.) were also active against the isolates and inhibited the growth of 90, 59.5 and 48.7% of the isolates respectively.

Reddy et al. [39] studied the antibacterial activity of the pure isolates from *Piper longum* (L.) (black pepper) and *Taxus bacata* (L.) (Yew). Three isolates of black pepper were active against gram-positive bacteria and moderately active against gram-negative bacteria. They reported that each isolate was highly active against at least one particular species of bacteria; piperlongumine against *Bacillus subtilis*, piperine against *Staphylococcus aureus,* and pellitorine against *Bacillus sphaericus*. 3-(3',4',5'-Trimethoxyphenyl) propionic acid did not show any bacterial activity. From the results they showed that most of the isolates of *Piper longum* had antibacterial activity.

Samy and Ignacimuthu [40] reported the antifungal activity of crude drug from the tree bark of *T. arjuna* which was tested against bacteria using the hole-plate diffusion method with concentrations of 5-25 mg/mL. The effective results of bacteria were confirmed by the dilution method (1.25-2.0 mg/mL) in MIC. The results were supported by pathochemical analysis. The specific activity against pathogenic bacteria, *Bacillus subtilis* and *Staphylococcus aureus* showed the traditional usage of the bark of *T. arjuna.*

Khan et al. [41] fractionated extracts of leaves, stem bark and root bark of *Eupomatia laurina* and performed test against 13 gram-positive and 12 gram-negative bacteria, a protozoan and four fungi. They found that all the extracts were active against

most of the bacteria and fungi and the dichloromethane and ethyl acetate extracts of the stem bark and the dichloromethane extract of the root bark exhibited superior levels of antibacterial activity.

Ramesh et al. [42] isolated friedelin, epi-friedelin, n-octacosanol, α-amyrin, sitosterol, sitosterol-3-D-glucopyranoside and luteoforol from *Bridelia crenulaa* Roxb. The aqueous and methanolic extracts and their fractions were tested against ten human pathogenic bacteria and four fungal strains. They observed that inhibitory activities were maximum in the chloroform-methanol (1:1) fraction of the methanolic extract against *E. coli, K. pneumoniae* and *P. aeruginasa*, which were responsible for the pathogenesis of urinary tract infection. The above study provided scientific evidence for the efficacy of the use.

Murillo-Alvarez et al. [43] extracted compounds from plants used in the traditional medicine of Baja California sur (Mexico) using ethanol as a solvent. They also tested antimicrobial activities of the isolated compounds. The antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Streptococcus faecalis, Candida albicans* and *Escherichia coli* were determined and *Aristolochia monticola, A. brevipes, Hymenoclea sp.* were found to be the most active.

Smith et al. [44] performed influence of medium type inoculums density and a cold incubation on antimicrobial assay sensitivity test. The largest and most distinct zones were produced using nutrient agar and the $1/10^4$ inoculum density for *Pseudomonas aeruginasa* and *Escherichia coli.* The greatest number of zones was detected without cold incubation. Using this method eight plants from Belize were screened for antibacterial activity. They reported that six plant extracts showed activity against the four organisms tested. Both inoculums density and medium type played important roles in assay sensitivity.

Srikrishna et al. [45] carried out antibacterial activity using cup plate method. They observed that petroleum ether, chloroform, methanol and water extract of the bark of *Aporosa lindleyana* (Euphorbiaceae) showed moderate to very good activity against bacteria such as *Bacillus subtilis, Escherichia coli.* They studied antifungal activity against *Penicillium chrysozenous, Candida albicans, Aspergilus niger* and *Trichoderma*

vridar and compared with the standard drug fluconazole. The petroleum ether extract showed considerable activity towards all the four fungal organisms.

Akinpelu [46] observed that 60% methanolic extract of the bark of *Anacardium occidentale* exhibited antimicrobial activity against 13 out of 15 bacterial isolates at a concentration of 20 mg/ml.

Audu et al. [47] extracted components from *Annona senegalensis* (root), *Nauclea latifolia* (stem bark) and *Ziziphus abyssinica* (root bark) using methanol, diethyl ether and cold water as solvent. They studied their activity on *Candida albicans*, *Escherichia coli, Salmonella sp.* and *Staphylococcus aureus* at different concentrations and found that all these components inhibited the growth of microbes.

Kamalakanman et al. [48] extracted 20 plant leaves and screened their inhibitory effect against the rice blast pathogen. They reported that *Prosopis juliflora* followed by *Zizyphus jujube* and *Abutilon indicum* significantly inhibited the mycelial growth and biomass as well as toxin production and spore germination under laboratory conditions.

Mehmood et al. [49] studied the antimicrobial potential of some Indian medicinal plants and their formulations. They tested twenty five different formulations based on five alcoholic extracts against several pathogenic micro-organisms. They observed that ten formulations showed higher potency compared to their constituents and good synergistic activity leading to significant reduction in the MIC values.

Ragasa et al. [50] extracted the air dried leaves of *Vitex negundo* which afforded vitexilactone and casticin by silica gel chromatography. Their structures were elucidated by extensive 1D and 2D NMR spectroscopy. They studied their activity and found to inhibit the growth of the fungi: *Candida albicans* and *Aspergillus niger* and the bacteria: *Staphyllococcus aureus* and *Pseudomonas aeruginasa*, but found inactive against *Escherichia coli* and *Bacillus subtilis*.

Habtemariam and Macpherson [51] investigated the cytotoxic and antibacterial activity of an ethanol extract of leaves of a herbal drug *Eupatorium perfoliatum*. They observed that the extract showed a potent cytotoxicity and weak antibacterial activity against gram-positive test organisms *Staphylococcus aureus* and *Bacillus megaterium*.

Mackeen et al. [52] reported that the crude ethanol extracts exhibited predominantly antibacterial activity with the root extract showing the strongest inhibition against the test bacteria at a minimum inhibitory dose (MID) of15.6 microg/disc. They observed that most of the extracts failed to inhibit the growth of fungi but the root, leaf, trunk and stem bark extracts showed strong antioxidant activity. Antitumour-promoting activity was shown by the fruit, leaf, stem, and trunk bark extracts.

Lall and Meyer [53] observed that the water and acetone extracts of roots of *Euclea natalensis* inhibited the growth of *Bacillus cerus, Bacillus pumilus, Bacillus subtilis, Micrococcus kristinae* and *Staphylococcus aureus* at concentration ranging between 0.1 and 6.0 mg/mL. They found that the water extract did not exert any inhibitory action on gram-negative bacteria while the acetone extract showed inhibitory activity at a concentration of 5.0 mg/mL against all the gram-negative bacteria investigated. The antibacterial activity of acetone extract was also investigated by a direct bioassay on TLC plates against *S. aureus.*

Pichai et al. [54] extracted the leaves of *Tabebuia rosea* using n-hexane, chloroform and aqua as solvents and screened the antibacterial activities against the pathogens *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* by agar dilution method. They observed that the aqueous extract exhibited potential antibacterial activity against *E. coli, S. typhi* and *S. aureus.* The hexane extracts had no effect on these three bacteria.

Ahmad and Beg [55] extracted 45 Indian plants traditionally used in medicine using ethanol as a solvent and studied their antimicrobial activity against certain drug-resistant bacteria and a yeast *Candida albicans.* They observed that 40 plant extracts showed varied levels of antimicrobial activity against one or more tested bacteria.

Savikin et al. [56] investigated the antimicrobial activity of the methanolic extracts of flowers and leaves of *Gentiana lutea* (L.) together with the isolated compounds mangiferin, isogentisin and gentiopicrin. They studied the activity against a gram-positive and a gram-negative bacteria as well as the yeast *Candida albicans* and

observed that both extracts and isolated compounds showed antimicrobial activity with MIC values ranging from 0.12-0.31 mg/mL.

Al-Hussaini and Mahasneh [57] studied the antimicrobial and antiqurorum sensing activities of fourteen ethanolic extracts of different parts of eight plants against four gram-positive, five gram-negative bacteria and four fungi. They recorded variable activities depending on the plant part extract and microorganism at 3 μg/disc. They found that among the gram-positive bacteria tested, the activities of *Laurus nobilis* bark extract ranged between a 9.5 mm inhibition zone against *Bacillus subtilis* up to a 25 mm one against methicillin resistant *Staphylococcus aureus*. They also found that *Staphylococcus aureus* and *Aspergillus fumigatus* were the most susceptible among bacteria and fungi tested towards plants parts. However, minimum inhibitory concentrations (MIC's) for both bacteria and fungi were relatively high (0.5-3.0 mg).

Ettebong and Nwafor [58] studied the antimicrobial activities of n-hexane, chloroform, ethyl acetate and methanol extract of *Carpolobia lutea* root which were used as a folk medicine in southern Nigeria against four typed cultures of bacteria namely, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis* and two clinical strains of fungi, namely, *Candida albicans* and *Tinea capitis* using agar well diffusion method. They reported that the ethyl acetate extract gave the widest zone of inhibition (21.0 mm) followed by chloroform when tested on *E. coli*. They also reported that none of the extracts showed any inhibitory effect against *Pseudomonas aeruginosa* and the fungal strains of *Candida albicans* and *Tinea capitis* and the most potent of these extracts was chloroform extract with minimum inhibitory concentration (MIC) of 25 mg/mL for bacteria. The phytochemical screening of the root of *C. lutea* revealed the presence of saponins, anthraquinones, flavonoids, cardiac glycosides, simple sugar and terpenes.

Alves et al. [59] evaluated the antimicrobial, antifungal and antiadherent activity of aroeira-do-sertao, mallow and guava tree on oral biofilm microorganisms and oral *candidiasis in vitro*. They found that the extracts were shown to be effective in inhibiting the growth of bacteria of the oral biofilm and fungi of oral *candidiasis*.

Qadrie et al. [60] studied the antibacterial activity of the ethanolic extract of *Indoneesiella echioides* (L.) by filter paper disc method. This method was based on the diffusion of an antibiotic from a filter paper disc through the solidified culture media of a petri dish. They observed that the growth was inhibited entirely in a circular area "zone around the filter paper" disc containing a solution of antibiotic and the plant extract. The used microorganisms were: *Staphylococcus aureus* and *Escherichia coli* and the organisms were maintained on nutrient agar slants. They tested the organisms using nutrient broth, one loop full of the respective cultures was taken in slants which were inoculated below 40^0C and incubated at 37^0C for 24 hrs and observed the growth with naked eye for their turbid nature and compared with that of sterile broth.

Duraipandiyan et al. [61] studied the antimicrobial activity of 18 ethnomedicinal plants collected from Palni hills of Southern Western Ghats against nine bacterial strains (*Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Ervinia sp., Proteus vulgaris*) and one fungal strain (*Candida albicans*) using paper disc diffusion method. They reported that out of 18 plants, 10 plants exhibited antimicrobial activity against one or more of the tested microorganisms at three different concentrations of 1.25, 2.5 and 5 mg/disc. The study evaluated the antimicrobial activity some of the ethnomedicinal plants used in folkloric medicine.

Gangoue-Pieboii et al. [62] investigated the antimicrobial activities *in vitro* of 10 plant species (*Voacanga africana, Crepis cameroonica, Plagiostyles africana, Crotalaria retusa, Mammea africana, Lophira lanceolata, Ochna afzelii, Ouratea elongate, Ou. flava* and *Ou. sulcata*) each of which used in the traditional medicine in Cameroon. They studied the activities of methanol extract of each plant in disc diffusion assays against 37 species or laboratory strains of seven species of microorganism (*Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus hirae, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Candida albicans).* They observed that each of the 10 methanol extracts displayed some degree of antimicrobial activity against at least one species of microorganisms and no activity was found against the gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae,*

Pseudomonas aeruginosa) and *Plagiostyles africana* showed greatest antimicrobial activity.

Yasunaka et al. [63] studied the antibacterial activity of thirty two extracts from 22 Mexican medicinal plants of 15 different families against *Escherichia coli and Staphylococcus aureus.* They reported that seventeen plants showed antibacterial, while five plants showed no activity against both bacteria and all of the extracts showed higher activity against *Staphylococcus aureus* than *Escherichia coli* except one.

Kumar et al. [64] carried out antimicrobial properties of a series of 61 medicinal plants belonging to 33 different families used in various infectious disorders at 1000 and 500 microg/mL concentration by agar dilution method against *Bacillus cereus, Bacillus pumilus, Bacillus subtilis, Bordetella bronchiseptica, Micrococcus luteus, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Streptococcus faecali, Candida albicans, Aspergillus niger and Saccharomyces cerevisiae.* They found that 28 plant extracts showed activity against at least one of the test organisms used. The crude extracts of *Dorema ammoniacum, Sphaeranthus indicus, Dracaena cinnabari, Mallotus philippinessis, Jatropha gossypifolia, Aristolochia indica, Lantana camara, Nardostachys jatamansi, Randia dumetorum* and *Cassia fistula* exhibited significant antimicrobial activity and property that support the folkloric use in the treatment of a broad-spectrum microbial effects.

Adamu et al. [65] carried out a survey of medicinal plants used locally in the treatment of various diseases in Bauchi State-Nigeria and total 84 medicinal plants were listed. They investigated the antimicrobial activity of the aqueous extracts of the plants and found that out of 84 plants, 75 exhibited antimicrobial activity against one or more of the test organisms at a concentration of 200 mg/mL. They found that the extracts showed potentially interesting activity against *Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli.*

Bonjar [66] studied the antibacterial activities of the 45 species of 29 plant families used in the traditional medicine by Iranian people against *Bacillus cereus,*

Bacillus pumilus, Bordetella bronchiseptica, Escherichia coli, Klebsiella pneumoniae Micrococcus luteus, Pseudomonas aeruginosa, Pseudomonas fluorescens, Serratia marcescens, Staphylococcus aureus and Staphylococcus epidermidis. He found that no plant showed activity against *Serratia marcescens and Bordetella bronchiseptica.* All the extracts showed the same activity 18 months later.

Saleh et al. [67] isolated the known triterpenoids lantic acid, camarinic acid and lantanilic acid from *Lantana camara* (L.) cultivated in Egypt and carried out the antibacterial activity of lantic acid using bioautography assays for gram-positive and gram-negative bacteria. They found that lantic acid possesses strong antibacterial activity against *Escherichia coli* and *Bacillus cereus* in which 0.08 and 0.1 μg were the minimum inhibition doses compared to 0.05 and 0.005 μg for chloramphenicol. The results showed that lantic acid has broad spectrum antibacterial activity and may hold potential as a non-selective antimicrobial agent.

Mathabe et al. [68] isolated four known compounds from the stem bark of *Spirostachys africana* using ethanol as a solvent which is used traditionally for the treatment of diarrhoea and dysentery in Limpopo province of South Africa. The isolated compounds were the two triterpenoids- d-friedoolean-14-en-oic acid-(3-acetyl aleuritolic acid) and lupeol, and two diterpenes- ent-2,6-α-hydroxy-norbeyer-1,4,15-trien-3-one (a diosphenol) and ent-3-beta-hydroxy-beyer-15-ene-2-one. They tested the antibacterial activity using micro dilution method and found that the frist triterpenoid as stated above, exhibited MIC of 50 microg/mL against *Staphylococcus aureus, Salmonella typhi, Vibrio cholera, Escherichia coli* and *Shigella dysentery* and the second one was not active against all tested microorganisms at 200 microg/mL and at 200 microg/mL all four compounds were not active against *Shigella sonnei.*

Angeh *et al* [69] isolated four known triterpenoids, 1α,3β-dihydroxy-12-oleanen-29-oic acid, 1-hydroxy-2-olean-30-oic acid, 3,30-dihydroxy-12-oleanen-22-one, and 1,3,24-trihydroxy-12-olean-29-oic acid along with a new pentacyclic triterpenoid (1α,23-dihydroxy-12-oleanen-29-oicacid-3β-O-2,4-di-acetyl-L-rhamnopyranoside) through a bioassay-guided procedure from the leaves of *Combretum imberbe*. They found that all the isolated compounds had moderate (62 μg/mL) to strong

(16 μg/mL) antimicrobial activity (MIC values) against *Staphylococcus aureus*, *Escherichia coli*, and compound 1α,3β-dihydroxy-12-oleanen-29-oic acid and 1α,23-dihydroxy-12-oleanen-29-oicacid-3β-O-2,4-di-acetyl-L-rhamnopyranoside were most active. The results of study gave credence to the ethnomedicinal use of *Combretum imberbe* and biological activity of its metabolities.

Mothana et al. [70] studied the antiproliferative activity against three human cancer cells, antimicrobial activity against antibiotic susceptible three gram-positive, three gram-negative bacteria and one fungal strain and three multiresistant *Staphylococcus* strains by the agar diffusion method and the determination of MIC against three gram-positive bacteria with the broth micro-dilution assay, as well as for their antioxidant activity using the DPPH radical scavenging method of sixty four methanolic and aqueous extracts of thirty Yemeni plants used in traditional medicine. They found that 12 plants showed growth inhibitory effect against all cancer cells with IC_{50} values< 50 μg/mL, 9 plants showed pronounced antimicrobial activity against gram-positive bacteria (including some multiresistant bacteria) with inhibition zones>15 mm and MIC values < 500 μg.

Shai et al. [71] isolated four compounds lupeol, betulinic acid, ursolic acid and 2α-hydroxyursolic acid from the leaves of *Curtisia dentate*. They studied the antibacterial and antifungal activity using broth microdilution assay and bioautography method and found that betulinic acid, ursolic acid and 2α-hydroxyursolic acid appreciably inhibited fungal growth with MIC values ranging from 8 to 63 μg/mL.

Khan et al. [72] extracted the leaves, seeds, stem and root barks, stem and root heart-woods of *Michelia champaca* using methanol, petroleum ether, dichloromethane, ethyl acetate, butanol as a solvent and observed that different fractions exhibited antibacterial activity. They also observed that fractionation drastically enhanced the level of activity particularly in all fractions of the stem bark and dichloromethane fraction of the root bark and some fractions of the leaves stem and root barks demonstrated activity against some of the tested moulds. They found that among all the fractions liriodenine was the active constituent of the root bark, with a broader and in some cases, better level of activity as compared to the standard.

Khan and Omoloso [73] extracted different fractions from the leaves, stem and root barks of *Dracantomelon dao* using methanol, petrol, dichloromethane, ethyl acetate, butanol as a solvent and found that they demonstrated a very good level of broad spectrum antibacterial activity. They reported that the dichloromethane and butanol fractions of the leaves were the most active and they had antifungal activity.

Khan and Omoloso [74] reported that the methanolic extracts and the fractions (petrol, dichloromethane, ethyl acetate and butanol) obtained from the leaves, seeds, stem and root barks of *Sarcocephalus coadunatus* exhibited a high level of broad spectrum antibacterial activity. They found that the activity was more pronounced in the dichloromethane; ethyl acetate and butanol fractions of the leaves; ethyl acetate and butanol fractions of the seeds; dichloromethane fractions of the stem bark and the ethyl acetate fractions of the root bark. None of the fractions showed any antifungal activity.

Dulger et al. [75] extracted compounds from three *Verbascum* (L.) species (*Verbascum olympicum* Boiss., *Verbascum prusianum* Boiss., and *Verbascum bombyciferum* Boiss.) and investigated their antimicrobial activity using the agar disc diffusion assay against *Escherichia coli* ATCC 11230, *Micrococcus luteus* La 2971, *Staphylococcus aureus* ATCC 6538P, *Salmonella thyphi* ATCC 19430, *Klebsiella pneumonia* UC57, *Pseudomonas aeroginosa* ATCC 27893, *Corynebacterium xerosis* CCM 2824, *Bacillus cereus* ATCC 7064, *Bacillus megaterium* DSM 32, *Mycobacterium smegmatis* CCM 2067, *Proteus vulgaris* ATCC 8427, *Candida albicans* ATCC 10231, *Rhodotorula rubra*, and *Saccharomyces cerevisiae* ATCC 9763. They found that *Verbascum* (L.) species showed antimicrobial activity against the gram-positive bacteria and yeasts, but no activity was seen against the gram-negative bacteria used in this study.

Kirmizigul et al. [76] reported antimicrobial and antifungal activities of the MeOH extract from the flowers of *Cephalaria transsylvanica* and three triterpenic acid glycosides, transsylvanoside A-C by MeOH using an agar-disc diffusion method. They observed that both the MeOH extract and the glycosides possess antimicrobial and antifungal activities against *Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Corynebacterium xerosis, Klebsiella pneumonia, Candida sutilis, Kluyveromyces fragilis, Aspergillus oryzae* and *Aspergillus flavus*.

Sudharmini and Ashalatha [77] isolated triterpenoids from *Myxopyrum smilacifolium* leaf and found the presence of ursolic acid (0.175 mg/g). They reported that the tritrpenoids showed antimicrobial activity in gram-positive bacteria and *Candida sps.*

Horiuchi et al. [78] isolated the effective compound and identified it as oleanolic acid, a triterpenoid from *Salvia officinalis* (Sage) leaves and tested antimicrobial activity against vancomycin-resistant enterococci (VRE). They also tested the antimicrobial activity of similar triterpenoids, ursolic acid, uvaol, betulinic acid and betulin and found that ursolic acid also showed antimicrobial activity against VRE. The minimum inhibitory concentrations (MICs) of oleanolic acid and ursolic acid were 8 and 4 μg/mL, respectively and these two compounds also showed antimicrobial activity against *Streptococcus pneumonia* and methicillin-resistant *Staphylococcus aureus* (MRSA). They also found that the compounds showed anti bactericidal activity against VRE at least for 48 h when added at concentrations that were two-times higher than their MICs.

Khan et al. [79] isolated amblyone, a triterpenoid from *Amorphophallus campanulatus* and studied in vitro antibacterial, antifungal and cytotoxic activities using disc diffusion technique and minimum inhibitory concentration was determined using serial dilution technique. They observed large zones of inhibition in disc diffusion antibacterial screening against four gram-positive bacteria (*Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus* and *Streptococcus pyogenes*) and six gram-negative bacteria (*Escherichia coli, Shigella dysenteriae, Shigella sonnei, Shigella flexneri, Pseudomonas aeruginosa* and *Salmonella typhi*) and the MIC values against these bacteria ranged from 8 to 64 μg/mL. In antifungal screening, the compound showed small zones of inhibition against *Aspergillus flavus, Aspergillus niger, Rhizopus aryzae*, and *Candida albicans.*

Leite et al. [80] obtained various organic and aqueous extracts from the leaves of *Indigofera suffruticosa* Mill (Fabaceae) by infusion and maceration and screened their antibacterial and antifungal activities. They were tested the extracts against 5 different species of humanpathogenic bacteria and 17 fungal strains by the agar-

solid diffusion method. They observed that most of the extracts were devoid of antifungal and antibacterial activities, except the aqueous extract of leaves of *I. suffruticosa* obtained by infusion, which showed strong inhibitory activity against the gram-positive bacteria *Staphylococcus aureus* with a minimal inhibitory concentration (MIC) of 5000 µg/mL. The MIC values to dermatophyte strains were 2500 µg/mL against *Trichophyton rubrum* (LM-09, LM-13) and *Microsporum canis*.

Mbwambo et al. [81] extracted compounds from stem bark, wood and whole roots of *Ternimalia brownii* using solvents of increasing polarity, namely, petroleum ether, dichloromethane, dichloromethane: methanol (1:1), methanol and aqua, and the extracts were tested for antifungal and antibacterial activity. They observed that the extracts of the stem bark, wood and whole roots of *T. brownii* exhibited antibacterial activity against standard strains of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhi* and *Bacillus anthracis* and the fungi, *Candida albicans* and *Cryptococcus neoformans*. They found that aqueous extracts exhibited the strongest activity against both bacteria and fungi.

Escalante et al. [82] isolated three monodesmosidic triterpenoid saponins from the butanolic extract of *Phytolacca tetramera* and established their structures. They reported that the three saponins belong to the olean-type triterpenoid saponins, with 28, 30-dicarboxylic groups and an olefinic double bond on C-12. They observed that phytolaccosides B and E showed antifungal activities against a panel of human pathogenic opportunistic fungi but phytolaccoside F did not show any activity. The most sensitive fungus was *Trichophyton mentagrophytes*.

Ofodile et al. [83] extracted compounds from the four species of *Ganoderma* available in Nigeria using n-hexane: diethyl ether, chloroform: acetone and methanol as a solvent and tested their antimicrobial activity. They found that all the three solvent extracts of all the species of *Ganoderma* were active against *Pseudomonas syringae* and *Bacillus subtilis*, whereas none of the extracts were active against *Cladosporium herbarum*.

Bouzada et al. [84] isolated 44 methanol extracts from 37 Brazilian traditional medicinal plants and evaluated for their antibacterial activity and toxicity to brine

shrimp using agar-well diffusion method against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhimurium, Shigella sonnei* and *Bacillus cereus*. They subjected to serial dilution assay by the extracts for determination of the minimum inhibitory concentration (MIC) and reported that extracts of *Baccharis dracunculifolia, Cajanus cajan, Eugenia uniflora, Solanum palinacanthum* and *Solanum concinnum* presented strong antibacterial activity with MIC values below 10 µg/mL.

Khan et al. [85] extracted crude from the leaves, stem bark, stem heart wood, root bark and root heart wood of *Euroschinus papuanus* and isolated fractions on partitioning with petroleum ether, dichloromethane, ethyl acetate and butanol and studied antibacterial and antifungal activity. They observed that ethyl acetate fractions of the stem heart wood, dichloromethane of root bark and ethyl acetate of root heart wood demonstrated excellent antibacterial activity and butanol fractions of leaves; stem heart wood and root bark demonstrated antifungal activity.

Ramesh et al. [86] tested the antimicrobial efficiency of aqueous, methanol, chloroform and hexane extracts of *Swertia corymbosa* and noticed maximum inhibitory activity against *Staphylococcus aureus* and *Salmonella typhi.*

Khan and Omoloso [87] extracted the *Breynia cernua* leaves, stem and root barks and heart woods with petrol, dichloromethane, ethyl acetate, butanol and methanol which gave various fractions. They studied antimicrobial activity of these fractions and found that the best activity was exhibited by the methanol extract of the root bark followed by its butanol fraction and the dichloromethane fraction of the stem bark also demonstrated good activity.

Lauk et al. [88] investigated antifungal activity of methanolic extract and alkaloidal fraction of *Berberis aetnensis* against *Candida species*. They observed that the crude extracts were active against *Candida species* and this activity was higher than that of the alkaloidal fraction and berberine.

Aqueveque et al. [89] isolated a new biologically active triterpenoid favolon B from fermentation broths of *Mycena sp.* Strain 96180. They found that favolon B

showed antifungal activities against *Botrytis cinerea, Mucor miehei, Paecilomyces variotii* and *Penicillium notatum*. No activities were observed against bacteria and yeast.

Ragasa et al. [90] extracted the essential oil of *Cymbopogon citratus* (DC) Stapf. by the supercritical fluid extraction process and fractionation of the oil afforded cymbopogonol and citral. Antimicrobial tests on cymbopogonol and citral indicated that they had moderate activity against *C. albicans* and low activity against *P. aeruginosa, E. coli, S. aureus* and *T. mentagrophytes* and both compounds were inactive against *B. subtilis* and *A. niger*.

From the above literature work it is evident that no systematic study has so far been carried out on the biocidal activity neither of the phytoconstituents isolated from the medicinal plants available in this region of West Bengal nor of their reported derivatives prepared so far. Thus it was felt necessary to include a systematic study of the biocidal activity of the phytoconstituents isolated from the medicinal plants available in this region as well as on their derivatives prepared by the use of different reagents.