SUMMARY AND CONCLUSION

The associated disadvantages with insulin and oral hypoglycaemic agents have led to stimulation in the research for locating natural resources showing antidiabetic activity. The traditional healers are using *Careya arborea* Roxb. and *Bridelia retusa* Spreng. in the treatment of hyperglycemia. A survey of the literature reveals that not much scientific evaluation has been conducted to check the antidiabetic potential of *Careya arborea* Roxb. and *Bridelia retusa* Spreng. This has prompted us to evaluate these drugs scientifically for their antidiabetic property.

In the present study, the barks of *Careya arborea* Roxb. family Lecythidaceae and *Bridelia retusa* Spreng, family: Euphorbiaceae were selected for pharmacognostical, phytochemical and pharmacological evaluation for possible antidiabetic activity.

The Plants were authenticated by the botanist Dr. U. S. Yadav, H. O. D. botany department, Willingdon College, Sangli and Dr. S. S. Sathe, botanist, Padmabhushan Dr. Vasantdada Patil Mahavidyalaya, Tasgaon. The voucher specimen has been preserved in our laboratory for future reference. The authenticated barks were subjected to organoleptic evaluation. Microscopic evaluation was carried out by studying the transverse section of the barks as well as observing the microscopical powdered characteristics of the bark. Different staining reagents were used to study transverse section and microscopic characteristics of powdered bark.

The barks were subjected to size reduction to get coarse powder (40#). Powdered material of the barks were used for physical evaluation. Physical evaluation comprised of different ash values like total ash, sulphated ash, water soluble ash, acid insoluble ash and extractive values viz. water soluble and alcohol soluble extractives. Moisture content was determined by three methods a) Loss on drying b) Azeotropic distillation and c) Karl Fischer titration method. The values have been reported elsewhere in the result and discussion. Possible microbial contaminations for the presence of *Escherichia. Coli, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus* were also carried out. It was found that all these microorganisms were absent in both the powdered drugs. The organoleptic, microscopical and physico-chemical parameters presented can be proposed as parameters to establish the authenticity of the said plants.

The standardised bark powders were subjected to successive extraction with various solvents like pet. ether (60-80°C), benzene, chloroform, acetone, ethanol by using soxhlet apparatus. Aqueous extraction was carried out using chloroform water (10%) I.P. by simple maceration method for seven days at room temperature. Powdered barks were
also extracted with alcohol using soxhlet apparatus. After effective extraction, solvent was distilled off by using rotary vacuum evaporator. The concentrated extracts were used for carrying phytochemical investigation, anti-diabetic activity and anticancer activity.

All the extracts were subjected to detailed preliminary phytochemical investigation. The phytochemical investigation revealed the presence of sterols, triterpenoids, flavonoids and phenolic compounds as a major active chemical constituents.

All the extracts were studied for acute oral toxicity study using OECD guidelines as well as Miller and Trainter method. Preliminary oral $LD_{50}$ values for pet. ether, benzene, chloroform, ethanol and alcoholic extracts of *Careya arborea* Roxb. were found to be 3000 mg/kg while 2000 mg/kg for acetone extract and 5000 mg/kg for aqueous extract. $LD_{50}$ values for pet. ether, benzene, chloroform, acetone, ethanolic extracts of *Bridelia retusa* Spreng. were found to be 2000 mg/kg while 3000 mg/kg for alcoholic extract and 5000 mg/kg for aqueous extract. 1/10$^{th}$ of this $LD_{50}$ was taken as effective dose (therapeutic dose) for subsequent studies.

The antidiabetic activity was performed using wistar albino rats. The animals were divided into ten groups, each group with six animals, two groups were selected as control, one as normal control and the other as diabetic control. One group was selected as a standard and the remaining were selected for the extracts: pet. ether, benzene, chloroform, acetone, ethanol (successive), alcohol and aqueous extracts.

By observing the results of acute antidiabetic study of *Careya arborea* Roxb., it can be concluded that the alcoholic extract, aqueous extract and pet. ether extracts had shown more prominent antidiabetic activity at the end of 6$^{th}$ hour, while benzene, chloroform, acetone extracts failed to produce significant antidiabetic activity. The chronic antidiabetic study for all the above extracts had shown more prominent antidiabetic activity at the end of 21$^{st}$ day, while benzene, chloroform and acetone extracts failed to produce antidiabetic activity. In acute study the alcoholic, aqueous and pet. ether extracts of *Bridelia retusa* Spreng. showed more prominent antidiabetic activity at the end of 4$^{th}$ hour and significant hypoglycemia was maintained for another two hours, while benzene, chloroform, acetone extracts failed to produce significant antidiabetic activity. The chronic study of *Bridelia retusa* Spreng. was also carried out for alcoholic and aqueous extracts which showed more prominent antidiabetic activity at the end of 21$^{st}$ day, while pet. ether, benzene, chloroform, acetone and ethanolic extracts failed to produce significant antidiabetic activity.
The relationship between blood glucose levels after an external load of glucose was studied using OGTT. In both cases, the blood glucose value in the control rats achieved a peak value at 60 minutes after glucose load and decreased to near normal levels at 120 minutes. While in diabetic control rats, the peak increase in blood glucose concentration was observed at 60 minutes. In case of Careya arborea Roxb. the alcoholic extract, aqueous extract and pet. ether extract significantly decreased the blood glucose level after 30 minutes. While glibenclamide treated diabetic rats showed significant decrease in blood glucose level after 60 minutes. But in case of Bridelia retusa Spreng. the alcoholic and aqueous extract significantly decreased the blood glucose level after 60 minutes.

At the end of the treatment, blood was collected by direct cardiac puncture and serum was separated by centrifugation at 2500 rpm. Total cholesterol, HDL cholesterol and triglycerides were estimated by using standard kit obtained from Biolab diagnostics (I) Pvt. Ltd. Tarapur, Maharashtra. The total cholesterol and triglycerides were found to be significantly increased in diabetic control group as compared with normal control. Treatment with alcoholic extract, pet. ether extract and aqueous extract of Careya arborea Roxb. significantly attenuated the elevated total cholesterol and triglyceride levels as compared with diabetic controls. HDL cholesterol level decreases in diabetic control group while all the above extracts significantly increases the HDL cholesterol level as compared with diabetic control. Treatment with alcoholic and aqueous extract of Bridelia retusa Spreng. significantly attenuated the elevated total cholesterol and triglyceride levels as compared with diabetic controls. HDL level decreases in diabetic control group. Treatment with alcoholic extract significantly (P<0.05) increases the HDL level in comparison with diabetic control while aqueous extract as well as std. is non-significant (P>0.05) in nature.

The present study revealed that both the plant extracts can be successfully utilized for the management of diabetes due to their antidiabetic action. One of the therapeutic approaches for reducing postprandial hyperglycemia in patients with diabetes mellitus is to prevent absorption of carbohydrates after food intake. Alcoholic and aqueous extracts of Careya arborea Roxb. showed more α-glucosidase inhibitory activity as compared to pet. ether extract. Alcoholic and aqueous extracts of Bridelia retusa Spreng. also showed α-glucosidase inhibitory activity. This may be one of the mechanisms responsible for its antidiabetic activity. Histopathological study clearly revealed that the damaged pancreas
in alloxan-treated diabetic control rats were regenerated by glibenclamide as well as comparable regeneration was shown by alcoholic extract of *Bridelia retusa* Spreng. This might be the other mechanism for the alcoholic extract of *Bridelia retusa* Spreng.

Alcoholic, aqueous and pet. ether extracts of *Careya arborea* Roxb. and alcoholic as well as aqueous extract of *Bridelia retusa* Spreng. were screened for anti-cancer activity using 5- FU as standard drug. Anti-cancer activity of pet. ether extract of *Careya arborea* Roxb. at 1000 µg/ml was comparable to that of std. drug 5- FU at 10 µg/ml. Alcoholic extract of *Bridelia retusa* Spreng. at 1000 µg/ml showed 58.49% viability as compared with 43.16 % viability at 10 µg/ml for std. drug 5- FU.

After pharmacological studies, the active extracts were selected for detailed phytochemical investigation. Initially total phenol and total flavonoid content of active fractions were determined by folin-ciocalteu reagent method and aluminium chloride method respectively. The free radical scavenging capacity of the extracts were determined using DPPH method. The study also revealed that the anti-oxidant activity increases with increase in content of phenol and flavonoids in the said extracts.

Alcoholic, aqueous and pet. ether extracts showed highly potent activity which may be due to sterols, flavonoids and phenolic compounds as major chemical constituents. Pet. ether extract showed the presence of nine coloured (pinkish) spots in benzene: ethyl acetate (9.75:0.25) which were confirmed by HPTLC. After confirmation of their presence by TLC, extract was subjected for isolation of sterol by column chromatography. The column chromatography was developed by using graded solvent mixtures of petroleum ether, pet.ether : benzene (1:1) and chloroform. One of the fraction showed single spot and was identified as lupeol.

The content of lupeol in alcoholic and pet. ether extract were calculated by using HPTLC and was found to be 0.84% and 14.58 % respectively. Lupeol was also isolated from the extract and was subjected to HPTLC study and estimated with standard lupeol. UV spectrum of isolated compound exactly concord with standard lupeol. Futher IR, NMR as well as GC-MS study of isolated lupeol was recorded and gave satisfactory results for confirmation of the structure. The interpretation of IR, NMR and Mass spectra is shown elsewhere in result and discussion.

The flavonoids were identified by TLC and separated by column chromatography. Ethyl acetate fraction of alcoholic extract showed the presence of two yellowish spots with toluene: ethyl acetate: formic acid (7.2:2.4:1) as a solvent system having 0.11 and 0.20 as Rf values. After confirmation of their presence by TLC, they were subjected to
isolation by column chromatography. The column chromatography was developed by using graded solvent mixtures of toluene: ethyl acetate. One of the fraction was identified as quercetin. HPTLC study of ethyl acetate fraction of alcoholic extract showed the presence of quercetin (19.24%) in comparison with standard quercetin with 0.19 as Rf value. UV spectrum of isolated compound exactly concord with standard quercetin. Further IR, NMR as well as GC-MS study of isolated quercetin was recorded and gave satisfactory results for confirmation of the structure. The interpretation of IR, NMR and Mass spectra is shown elsewhere in result and discussion.

Alcoholic extract of Careya arborea Roxb. showed more potent antidiabetic activity as it contained steroidal triterpenes like lupeol, flavonoids like quercetin and phenolic compounds. Aqueous extract mainly contained flavonoids and phenolic compounds while pet. ether extract contained only steroidal triterpenes. Both extracts individually showed anti-diabetic activity. The higher potent activity of the alcoholic extract might comprise of the synergistic effect of triterpenes (steroidal), flavonoids and phenols.

Alcoholic extracts of Bridelia retusa Spreng. showed highly potent antidiabetic activity which may be due to presence of sterols, flavonoids and phenolic compounds as major chemical constituents. Alcoholic extract showed the presence of two coloured (pinkish) spots in toluene: methanol: formic acid (9: 1:0.5). After confirmation of the presence of sterol by TLC, the extract was subjected to isolation of sterol by column chromatography. The column chromatography was developed by using graded solvent mixtures of toluene and methanol. One of the fraction was confirmed as β–sitosterol. The HPTLC study of alcoholic extract showed the presence of β–sitosterol (0.67%) and was compared with standard β–sitosterol. Both the compounds showed similar Rf values. Isolated compound was also confirmed and estimated with standard β–sitosterol. UV spectrum of isolated compound exactly concord with standard β–sitosterol. Further IR, NMR as well as GC-MS study of isolated β–sitosterol was recorded and gave satisfactory results for confirmation of the structure. The interpretation of IR, NMR and Mass spectra is shown elsewhere in result and discussion.

HPTLC study of ethyl acetate fraction of alcoholic extract showed the presence of quercetine (21.71%) as compared with standard quercetin with 0.19 as Rf values for both. UV spectrum of isolated compound exactly concorded with standard quercetin. Further IR, NMR as well as GC-MS study of isolated quercetin was recorded and gave satisfactory results for confirmation of the structure. The interpretation of IR, NMR and Mass spectra is shown elsewhere in result and discussion.

Alcoholic extract of Bridelia retusa Spreng. contains mainly steroids like β–sitosterol as well as flavonoids and phenolic compounds which act synergistically and
may be responsible for the antidiabetic activity. Hence it can be concluded that ethanolic extract of bark of *Bridelia retusa* Spreng. exhibited significant antidiabetic activities in alloxan-induced diabetic rats. This extract showed improvement in parameters like body weight, lipid profile and \( \alpha \)-glucosidase inhibitor as well as regeneration of \( \beta \)-cell of pancreas and so might be of great value in the treatment of diabetes mellitus.