According to WHO, roughly two-thirds to three quarters of the world’s population relies upon medicinal plants for its primary health care. In search of novel biological activity and development of evidence based modern phytomedicine, the rich diversity of medicinal plants of the globe is under screening and evaluation.

India has huge diversity of medicinal plants with several known therapeutic properties. However, majority of rich diversity of medicinal plants are yet to be exploited for their biological potential. Recently, there is an increased quest to redesign the screening strategies to get novel or alternative therapeutic compounds or herbal preparation against various tropical diseases, complex chronic and infectious diseases including cancer or where modern medicine is not available or less effective. It is expected that traditionally used medicinal plants have least or no toxicity and may provide safe novel herbal preparation or compounds against target disease. Such areas of interest include the problems caused by MDR bacteria, non-effective treatment of several chronic diseases like cancer and neurodegenerative diseases etc.

In the past, Indian medicinal plants have been systematically screened for their several pharmacological properties including antimicrobial, antidiabetics, antimalarial, antioxidant, anticancer activities at many institutions including CDRI, Lucknow and others which showed varying level of in vitro and in vivo activities. However, concerted efforts for the systematic screening of medicinal plants for their broad spectrum antimicrobial activity against drug resistant pathogens, antioxidant and antimutagenic activities are less explored. Considering the importance of targeted screening for discovery of novel biological activities and their therapeutic potential, the plants have been selected which are a part of traditional system of medicine and are mainly used as spices. Since these plants are safe and non toxic in nature, hence expected to yield more useful data. Considering the importance and need for screening of medicinal plants, the present study has been taken with following objectives

1. To determine the antibacterial activity of certain Indian medicinal plants especially against drug resistant bacteria.

2. To screen the broad spectrum antioxidant activities of plant extracts by using different in vitro assays.
Summary

3. To evaluate the antimutagenic properties of certain antioxidant active plant extracts against direct and indirect acting mutagens using Ames Salmonella assay.

4. Phytochemical analysis of most bioactive fractions by different spectroscopic and chromatographic techniques.

The work done on the basis of above objectives is briefly summarized below.

Antibacterial activity of medicinal plant extracts

Antibacterial activity of methanolic extracts from 25 selected plants was evaluated against different bacteria (Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli and Salmonella typhimurium). The tested plant extracts showed varying level of antibacterial activity ranging from 11 to 34 mm inhibition of zone diameter. Several plants extract demonstrated broad spectrum activity and inhibited all the tested bacteria. S. aureus being the most sensitive to plant extracts. The sensitivity pattern of the reference strains to plant extracts used in the study was found in the decreasing order of S. aureus > B. subtilis > E. coli > S. typhimurium > P. aeruginosa. Methanolic extracts of twelve plants namely Allium sativum, Capsicum frutescens, Carum copticum, Cinnamomum zeylanicum, Coriandrum sativum, Murraya koenigii, Piper cubeba, Piper nigrum, Psidium guajava, Punica granatum, Syzygium aromaticum and Zingiber officinale inhibited the growth of all the five test bacteria and designated as most promising broad spectrum medicinal plants. However, three other plants extracts; Cuminum cyminum (fruit), Myristica fragrans (aril) and Myristica fragrans (seed) showed almost similar activity by inhibiting 80% of the test bacteria.

Further, all plant extracts were evaluated for their activity against two important problematic groups of multidrug resistant bacteria (MRSA and ESβL producing K. pneumoniae). The biochemical and antibiotic resistance behavior including presence of mecA gene in MRSA and production of ESβL have been tested to ensure the MDR behavior. Results indicated that active plant extracts are almost equally effective against both reference and multidrug resistant strains. Moreover, strain to strain variation was also evident in their activity.
Based upon their broad spectrum antibacterial activity nine plants belonging to most promising bioactive group were selected for further study which includes *Carum copticum* (fruits), *Cinnamomum zeylanicum* (bark), *Murraya koenigii* (leaves), *Piper cubeba* (fruits), *Piper nigrum* (seeds), *Psidium guajava* (leaves), *Punica granatum* (peel), *Syzygium aromaticum* (buds) and *Zingiber officinale* (rhizomes). Potency of these plant extracts was determined in terms of their minimum inhibitory concentration (MIC) against drug resistant pathogenic bacteria. The MIC values ranged from 0.4 to $\geq 3.2$ mg/ml against MRSA and ESβL producing *K. pneumoniae* strains. Seven plants were further selected for fractionation in organic solvents in order of their polarity viz. petrol ether, benzene, ethyl acetate, acetone, methanol and ethanol to obtain the most promising broad spectrum active fraction.

The respective fractions of seven plants namely *C. copticum*, *M. koenigii*, *P. cubeba*, *P. nigrum*, *P. guajava*, *P. granatum* and *Z. officinale* demonstrated varying level of antibacterial activity against MRSA and ESβL producing *K. pneumoniae* with zone of inhibition ranging from 10-34 mm. The MIC of selected plant fractions exhibiting broad spectrum activity which ranged from 0.4-3.2 mg/ml for *P. granatum*, 0.4 to $\geq 3.2$ mg/ml (*C. copticum*), 0.8 to $\geq 3.2$ mg/ml (*M. koenigii*), 1.6 to $\geq 3.2$ mg/ml (*Z. officinale* and *P. guajava*), $\geq 3.2$ mg/ml (*P. cubeba* and *P. nigrum*). Methanol fraction was found to be the most active fraction in *C. copticum*, *P. nigrum*, *P. guajava* and *P. granatum*. While benzene, ethyl acetate and ethanol were the most active fractions for *M. koenigii*, *Z. officinale* and *P. cubeba*. Overall promising antibacterial activity and potency of extracts was *P. granatum* (methanol) > *C. copticum* (methanol) > *M. koenigii* (benzene) > *Z. officinale* (ethyl acetate) > *P. guajava* (methanol) > *P. cubeba* (ethanol) > *P. nigrum* (methanol). Phytochemical analysis of some of these fractions revealed the presence of different major phytocompounds as described in subsequent section.

**Antioxidant activity of methanolic medicinal plant extracts**

All extracts from 25 plants under study were subjected to antioxidant scrutiny by four different antioxidant methods viz. DPPH free radical scavenging activity, reducing power activity by FRAP and CUPRAC assays and total antioxidant capacity by phosphomolybdenum method. Due to diversity of antioxidant phytocompounds in
plant extracts, use of more than one method is preferable to detect broad spectrum antioxidant activity. The extracts were tested at concentrations ranging from 12.5-400 \( \mu g/ml \). Of these twenty six extracts from 25 traditionally used medicinal plants, seventeen demonstrated strong (>70%) decolorization at 400 \( \mu g/ml \) dose by DPPH scavenging assay. The values were comparable to standards antioxidants (ascorbic acid and BHT). The remaining extracts showed good (50-70% decolorization) to weak free radical scavenging (<50% decolorization) activity. Similarly, by FRAP assay, 19 plant extracts demonstrated powerful ferric ions (Fe\(^{3+}\)) reducing ability (absorbance \( \geq 1.0 \)). The reducing power of all plant extracts increased with increasing concentration of the extracts. On the other hand, when these extracts were tested by cupric ion reducing antioxidant capacity (CUPRAC) method, eighteen plant extracts showed strong reducing power (absorbance \( \geq 1.0 \)).

Total antioxidant capacity calculated by phosphomolybdenum method revealed dose dependent, high total antioxidant capacity (\( \geq 1000 \mu mol \) of ascorbic acid/ g) in fifteen plant extracts at the concentration of 400 \( \mu g/ml \). The comparative analysis of medicinal plants extracts determined by all the four methods suggested that \( A. \ cepa, \ C. \ copticum, \ C. \ cyminum, \ C. \ longa, \ L. \ nobilis, \ M. \ koenigii, \ M. \ fragrans \) (aril), \( M. \ fragrans \) (seed), \( P. \ guajava, \ P. \ granatum, \ S. \ aromaticum, \ Z. \ officinale \) possessed broad spectrum antioxidant activity. Although few other plants extracts like \( C. \ frutescens, \ C. \ zeylanicum, \ C. \ sativum, \ F. \ vulgare \) and \( F. \ asafoetida \) also revealed fair antioxidant activity by two or more methods.

**Fraction based antioxidant activity of selected medicinal plants**

On the basis of broad spectrum antioxidant activity of medicinal plant extracts, five plants with strong antioxidant activity namely \( Carum \ copticum, \ Punica \ granatum, \ Murraya \ koenigii, \ Zingiber \ officinale \) and \( Psidium \ guajava \) and two plants, \( Piper \ cubeba \) and \( Piper \ nigrum \) showing relatively less activity were selected for fraction based antioxidant activity determination. The purpose of this study was to fractionate these plants and locate the most active constituents.

The fractions of \( C. \ copticum \) showed a varied level of free radical scavenging activity by DPPH assay. The methanol fraction was found to be the most active free radical scavenger followed by ethanol. The other fractions exhibited relatively less activity.
Summary

ranging from 3.8% to 20% decolorization. These fractions had effective reducing power using the potassium ferricyanide reduction method. Similarly, the antioxidant activity by CUPRAC assays indicated the highest reducing power potential in methanol fraction followed by ethanol and acetone fractions. The results are comparable to ascorbic acid and BHT. By phosphomolybdenum method, the C. copticum fractions exhibited concentration dependent antioxidant capacity with respect to ascorbic acid equivalents. At 100 μg/ml concentration, the methanol fraction showed maximum antioxidant capacity (2087.7 μmol) followed by ethanol fraction (1514.8 μmol).

Punica granatum (peel) fractions had concentration dependent free radical scavenging activity. DPPH absorption was inhibited maximum by methanol fraction followed by acetone, ethanol and ethyl acetate fractions. These fractions also had effective reducing power using the potassium ferricyanide reduction method. Similarly, the antioxidant activity by CUPRAC assays indicated the highest reducing power potential in methanol fraction followed by ethanol, acetone and ethyl acetate fractions. The results are comparable to ascorbic acid and BHT. Moreover, at highest tested concentration (80 μg/ml), the methanol fraction showed maximum antioxidant capacity followed by ethanol and acetone fractions as evident from phosphomolybdenum method.

Similarly, antioxidant activity of M. koenigii estimated by DPPH, FRAP, CUPRAC and phosphomolybdenum assays revealed maximum activity in benzene fraction followed by ethyl acetate and petrol ether fractions. The results are comparable with their respective controls at tested concentration of 12.5-100 μg/ml. Moreover, a concentration dependent activity was also observed in all the above assays.

The ethyl acetate fraction of Zingiber officinale showed remarkably higher degrees of radical scavenging (88.3%) while acetone fraction was the second most DPPH radical scavenger (84.0%) followed by methanol, ethanol, benzene and petrol ether fractions at tested concentrations of 10-80 μg/ml. It is evident that the ethyl acetate and acetone fractions had effective reducing power using FRAP method followed by other fractions. However, the antioxidant activity by CUPRAC assay indicated the highest reducing power potential in ethyl acetate fraction followed by acetone and methanol
fractions. Moreover, at highest tested concentration of 80 µg/ml, the ethyl acetate fraction showed maximum antioxidant capacity (4944.1 µmol) by phosphomolybdenum method followed by acetone, petrol ether, benzene, methanol and ethanol fractions.

By DPPH assay, a concentration dependent response is evident in all the fractions (methanol, acetone and ethanol) of *Psidium guajava* at tested concentrations of 10-80 µg/ml. Methanol showed 85% of scavenging followed by acetone, ethanol and ethyl acetate fractions. Similar trend was found for reducing power potential by FRAP and CUPRAC methods. Similarly, by phosphomolybdenum method, the methanol fraction showed maximum antioxidant capacity (4175.1 µmol) followed by ethanol fraction (1733.4 µmol) at highest tested concentration of 80 µg/ml.

The fractions of *P. nigrum* (seeds) showed poor (< 50.0% free radical scavenging) in all the fractions at tested concentrations of 25-200 µg/ml. Similarly, these fractions had ineffective reducing power using the potassium ferricyanide reduction method when compared to the standards. But the antioxidant activity by CUPRAC assays indicated the moderate reducing power potential in methanol fraction followed by ethanol and other fractions. However, total antioxidant activity was lower in all the fractions by phosphomolybdenum method.

Similarly the free radical scavenging activity of the fruits fractions of *P. cubeba* by DPPH method revealed that all the fractions were relatively less efficient as radical scavengers except ethanol fraction inhibiting 63.4% absorption of DPPH. These fractions had low reducing power using FRAP and CUPRAC method when compared to the standards. Total antioxidant activity by phosphomolybdenum method was also low in all the respective fractions at the tested concentrations of 25-200 µg/ml.

**Antimutagenic activity of antioxidant active fractions of tested medicinal plant extracts**

On the basis of antioxidant activity of various fractions of seven plants, most active antioxidant fraction of each plant was selected to evaluate their antimutagenic potentials. Two fractions from *P. nigrum* and *P. cubeba* which revealed relatively poor antioxidant activity had also been subjected to antimutagenic evaluation to assess the possible correlation between antioxidant and antimutagenic properties.
Therefore, based on promising antioxidant activity, the methanol fraction of *C. copticum, P. granatum, P. guajava* and *P. nigrum; benzene fraction of M. koenigii, ethyl acetate fraction of Z. officinale* and ethanol fraction of *P. cubeba* were evaluated for their antimutagenic activities by Ames test against direct acting (NaN₃ and MMS) and indirect acting (2-AF and B(a)P) mutagens. These fractions at respective tested concentrations by plate incorporation method could not show any sign of mutagenicity and toxicity to *Salmonella typhimurium* strains, either alone or in the presence of S9 mix.

Antimutagenic activity of methanol fraction of *C. copticum* was found to be dose dependent. At a dose of 100 μg/plate, antimutagenic response was significant at P < 0.05 against TA97a with a percent mutagenicity decrease of 73.9 followed by TA100 (70.4%), TA102 (56.0%) and TA98 (54.1%) strains against NaN₃ induced mutagenicity. Similar trend of activity was obtained against MMS induced mutagenicity where antimutagenicity varied from 58.9% to 83.1%. The methanol fraction at all doses was inhibitory (50.9% to 64.5%) for B(a)P induced mutation. The order of antimutagenic activity was found to be in order of *S. typhimurium* TA100 > TA98 > TA102 > TA97a. Likewise, antimutagenic activity of methanol fraction against 2-AF ranged from 55.4% to 68.5%. The significant reduction (P < 0.005) in number of revertants was recorded for TA98 followed by TA100, TA97a and TA102. The linear regression analysis between extract dose and antimutagenic response was found to be highly significant.

Methanol fraction of *P. granatum* (peel) when evaluated for its antimutagenic activity at 10, 20, 40 and 80 μg/plate showed concentration dependent activity. At the concentration of 80 μg/plate, the extracts exhibited maximum antimutagenicity in TA100 (84.5%) followed by TA97a (80.4%), TA98 (76.8%) and TA102 (66.8%) tester strains against NaN₃ induced mutagenicity. Likewise, the inhibition percent of 76.6% to 91.9% was recorded against MMS induced mutagenicity. The *P. granatum* was also evaluated against benzo(a)pyrene and 2-aminoflourene that infers mutagenicity by microsomal activation. The dose dependent antimutagenic response was highly significant against B(a)P with percent inhibition of mutagenicity ranged from 81.2% to 87.2%. Similar trend of antimutagenic activity against 2-AF was also shown by *P. granatum* methanol fraction. The significant reduction (P ≤ 0.05) in
number of revertants was recorded by TA100 (88.9%) followed by TA102 (86.0%), TA97a (83.8%) and TA98 (82.3%). The linear regression analysis between extract dose and antimutagenic response was also highly significant.

Similarly, the antimutagenic activity of benzene fraction of *M. koenigii* was found to be dose dependent (12.5, 25, 50 and 100 µg/plate). At a dose of 100 µg/plate, antimutagenic response was significant at $P < 0.05$ against TA97a with a percent mutagenicity decrease of 84.9% followed by TA100 (84.4%), TA98 (73.2%) and TA102 (72.2%) strains against NaN₃ induced mutagenicity. Likewise, the percent decrease in number of His* revertants was significant at $P < 0.005$ and ranged from 74.1% to 86.0% against MMS induced mutagenicity.

The benzene fraction was antimutagenic for B(a)P induced mutation at $P < 0.001$ and ranged from 80.1% to 86.0%. The antimutagenic activity was found in order of *S. typhimurium* TA102> TA100> TA97a> TA98. Similarly, trend in antimutagenicity has been shown by benzene fraction against 2-AF. Hence, *M. koenigii* demonstrated significant antimutagenesis against both base pair and frameshift mutations.

Similarly, ethyl acetate fraction of *Z. officinale* exhibited maximum antimutagenicity in TA100 (86.9%) followed by TA97a (86.7%), TA98 (75.7%) and TA102 (70.4%) against NaN₃ induced mutagenicity. The results were statistically significant at $P \leq 0.05$ except TA102 whilst the inhibition percent of MMS induced mutagenicity was recorded as 96.7% in TA100, 91.5% in TA102, 86.7% in TA97a and 62.1% in TA98. Similarly, the dose dependent antimutagenic response was highly significant ($P < 0.005$) against B(a)P with percent inhibition of mutagenicity ranged from 83.8% to 88.2%. Similar trend of antimutagenic activity against 2-AF was shown by *Z. officinale* ethyl acetate fraction. The significant reduction ($P < 0.005$) in number of revertants was recorded by TA100 (88.2%) followed by TA102 (86.3%), TA97a (84.5%) and TA98 (82.5%). All the strains demonstrated reduction in the revertants in a dose dependent manner with the regression values ranged from 0.97 to 1.0.

Methanol fraction of *P. guajava* leaves revealed concentration dependent antimutagenicity in Ames test. Guava had significant antimutagenic potential and inhibited NaN₃ induced revertants by 76.1-84.8% ($P \leq 0.05$) and MMS induced revertants by 73.9%-86.2% ($P < 0.005$). The antimutagenic effect of methanol
fraction was significant (P < 0.005) and found to be concentration dependent. The *P. guajava* methanol fraction when tested against benzo(a)pyrene and 2-aminoflourene in the presence of S9 revealed a dose dependent antimutagenic response (P < 0.001) with percent inhibition of mutagenicity ranged from 80.6% to 85.9%.

The methanol fraction of *P. nigrum* seeds at tested concentrations (25, 50, 100 and 200 μg/plate) was antimutagenic and found to be dose dependent. At a dose of 200 μg/plate, antimutagenic response was significant (P < 0.05) against TA97a and TA100 with a mutagenicity decrease of 66.8% and 68%, while it was non-significant for TA98 (61.5%) and TA102 (58.8%) against NaN₃ induced mutagenicity. However, against MMS induced mutagenicity the response was significant against all the strains except TA98. Moreover, methanol fraction had dose dependent response against B(a)P induced mutagenicity and it ranged from 62.4% to 68.4% (P < 0.005). Likewise, significant antimutagenic trend (P < 0.05) has been shown against 2-AF which ranged from 60.2% to 68.1%.

Different concentrations (25-200 μg/plate) of the *P. cubeba* ethanol fraction were taken for antimutagenicity assay. At the concentration of 200 μg/plate, the extracts exhibited 64.5% antimutagenicity in TA100 followed by TA97a (60.7%), TA102 (51.5%) and TA98 (46.2%) tester strains against NaN₃ induced mutagenicity. The results were statistically significant only for TA100. Likewise, the percent inhibition of MMS induced mutagenicity was recorded 64.9% in TA102, 60.4% (TA100), 55.1% (TA97a) and 50% (TA98). Moreover, the dose dependent antimutagenic response against B(a)P was highly significant (P ≤ 0.005) with percent inhibition of mutagenicity ranged from 59.5% to 66.2%. Similar trend of antimutagenic activity was shown against 2-AF where the significant reduction (P ≤ 0.05) in number of revertants was recorded for TA102 (65.0%) followed by TA100 (61.9%), TA97a (56.9%) and TA98 (56.4%). The regression values ranged from R² = 0.92 to 0.98.

**Antimutagenic activity of pure phytocompounds (Punicalagin and Ellagic acid)**

Based on promising antimutagenic activity of *P. granatum* (methanol fraction), their two major detected compounds viz. punicalagin and ellagic acid were evaluated for their antimutagenic potential by Ames test against direct acting (NaN₃ and MMS) and indirect acting (2-AF and B(a)P) mutagens. The compounds at selected concentrations
Summary

(50, 100, 250 and 500 μM/plate) by plate-incorporation method could not show any sign of mutagenicity and toxicity to Salmonella typhimurium strains, either alone or in the presence of S9 mix. The antimutagenic activity of both the compounds was dose dependent. At maximum dose (500 μM/plate) of ellagic acid, antimutagenic response was significant (P < 0.005) against TA97a with mutagenicity decrease of 72.1% followed by TA100 (65.9%), TA98 (64.2) and TA102 (62.3) strains against NaN3 induced mutagenicity. Similar trend of activity was obtained against MMS induced mutagenicity where percent decrease in number of His+ revertants was significant (P < 0.005) for TA102 (73.7%) followed by TA98 (69.0%), TA97a (66.5%) and TA100 (65.3%). The antimutagenicity of ellagic acid against indirect acting mutagens benzo(a)pyrene and 2-aminoflourene was significant at P < 0.005 and the dose dependent response of the antimutagenic behavior against respective mutagens was ranged from 78.6% to 88.9%.

Similarly, at a dose of 500 μM/plate of punicalagin, antimutagenic response was significant at P < 0.05 against TA97a with mutagenicity decrease of 74.4% followed by TA100 (74.3%), TA98 (65.3%) and TA102 (59.8%) strains against NaN3 induced mutagenicity. Similar trend of activity was obtained against MMS induced mutagenicity. The antimutagenicity of punicalagin against indirect acting mutagens benzo(a)pyrene and 2-aminoflourene was significant at P < 0.005 and the dose dependent response of the antimutagenic behavior against respective mutagens was ranged from 76.7% to 85.0%. Further, the linear regression analysis between extract dose and antimutagenic response was found to be significant.

Phytochemical analysis

The total phenolics concentration as gallic acid equivalents has been estimated in all the methanolic plant extracts by Folin-Ciocalteu method. The total phenolic content varied from 24.95 to 329.92 mg/g of extract. Some plant extracts namely A. cepa, C. frutescens, C. coticum, C. zeylanicum, C. longa, M. koenigii, M. fragrans (aril), P. guajava, P. granatum, S. aromaticum and Z. officinale showed high level of total phenolic content (≥ 150 mg/g of extract). The each most bioactive fraction of the selected seven plants was also investigated by color test and IR spectral analysis.

Phytochemical analysis of C. coticum fruit fractions revealed the presence of
alkaloids and phenolics as major groups of compounds. The total phenolic content of
dry extract and various fractions determined by the Folin-Ciocalteu method showed
highest polyphenolic content (119.2 ± 0.3 mg GAE/g) in methanol fraction followed
by other fractions. The HPLC analysis of methanol fraction of *C. copticum* revealed
the presence of various compounds at different wavelengths and different retention
times. However, thymol was found as the major constituent. The retention time of
thymol in aqueous phosphoric system was 49.5 min and constitutes second largest
peak in methanol. One major peak could not be identified in HPLC due to
unavailability of required standards. Four components were identified in GC-MS by
direct similarity searches for *C. copticum*. These numbers may be extended with the
help of chemometric techniques. The major compounds identified were thymol
(95.14%), linolelaidic acid, methyl ester (1.54%), cis, cis-linoleic acid (2.55%) and 3-
nitrophthalic acid (0.77%) respectively.

Phytochemical analysis of pomegranate (peel) revealed the presence of phenolics as
major group of compounds. The total phenolic content of various fractions (mg/g of
dry extract) was determined as gallic acid equivalents by the Folin-Ciocalteu method.
Methanol fraction contained 468.3±5.5 mg GAE/g of dry extracts followed by the
ethanol (414.6±5.9), acetone (219.3±1.1) and ethyl acetate (20.3±0.7) fractions.

The plant fractions which displayed fair to good antioxidant activity were subjected to
HPLC followed by LC-MS analysis. Interestingly, HPLC analysis of acetone and
methanol fractions confirmed the presence of punicalagins A and B as well as ellagic
acid; however the relative abundance of ellagic acid was more in methanol fraction as
compared to acetone fraction. The retention times of punicalagins A and B and ellagic
acid in aqueous phosphoric system were found to be 28.5 min, 30.5 min and 37.5 min
when compared with the required standards. It was not possible to identify the other
minor peaks in HPLC due to unavailability of standards.

The presence of various polyphenols can be seen in all the fractions characterized by
MS analysis. In addition to punicalagins and ellagic acid, punicalin, gallic acid and
few other phenolics are present in appreciable amount. LC-MS spectra by direct
infusion of *Punica* fractions shows the presence of punicalagins (*M – H m/z* 1083),
punicalin (*M – H m/z* 781), corilagin (*M – H m/z* 633), gallic acid (*M – H m/z* 601),
2.3-(S)-HHDP-D-glucose (M−H m/z 433), ellagic acid (M−H m/z 301). The other peaks of major compounds are also identified in *Punica granatum* fractions.

Phytochemical analysis of fractions revealed the presence of alkaloids, phenolics and glycosides as major group of compounds. The total phenolic content (mg/g) of *Murraya koenigii* various fractions showed highest polyphenolic content (187.1±6.3) in benzene fraction followed by petrol ether (146.4±6.3), ethyl acetate (113.9±3.0), acetone (113.9±2.7), ethanol (110.3±2.0) and methanol (103.2±3.1) fractions.

A total of 21 chemical components were identified in leaf extract by GC-MS analysis. These numbers may be extended with the help of chemometric techniques. The major compounds identified were caryophyllene (14.8%) followed by 3-undecen-5-yne (Z)-(9.52%), phytol (9.17%), 2-methyl-3H-phenanthro[3,4-D] imida (8.90%), caryophyllene oxide (6.61%), propylparaben (6.11%), D-limonene (6.01%). The remaining compounds were present in percentages of 1.06-5.72.

Ginger (rhizome) fractions revealed the presence of alkaloids and phenolics as major group of compounds. However, ethyl acetate contained the highest amount of phenolics (136.3±4.8 mg GAE/g). Phenolics were also higher in the acetone (120.9±3.7) followed by methanol (101.2±3.9), ethanol (85.0±2.1), benzene (73.6±3.0) and petrol ether (69.3±5.8) fractions.

The GC-MS analysis of *Zingiber officinale* ethyl acetate fraction revealed 12 chemical components which may be extended with the help of chemometric techniques. The major compounds identified were 3,6-dimethyl-2,3,3a,4,5,7a-hexahydro (23.69%), 1,3-cyclohexadiene, 5-(1,5-dimethyl (14.48%), gingerol (13.54%), benzene,1-(1,5-dimethyl-4-hexenyl (10.60%), cyclohexene,1-methyl-4-(5-methyl (10.40%), 1,3-cyclohexadiene,5-(1,5-dimethyl (8.07%). The other compounds were present in low percentages ranging from 0.67-4.27 respectively.

Phytochemical analysis of fractions revealed the presence of alkaloids, phenolics and glycosides as major group of compounds. The total phenolic content (mg/g) of *Psidium guajava* various fractions showed highest polyphenolic content (261.4±8.5) in methanol fraction followed by the ethanol (146.7±2.2), ethyl acetate (99.6±2.4), acetone (84.2±2.4), benzene (43.8±2.3) and petrol ether (41.2±1.9) fractions.
Ten different compounds were identified in GC-MS. *Psidium guajava* leaves extract contained 4-methylthiazole (14.29%), 13-tetradecenal (11.62%), 2-nonanone, 9-hydroxy- (10.48%), 2-butyne, 1, 4- dichloro- (7.10%). The remaining compounds (1.3-5.1% of total) were present in *P. guajava* methanol fraction.

Phytochemical analysis of fractions revealed the presence of alkaloids and phenolics as major group of compounds. The total phenolic content (mg/g) of *Piper nigrum* was maximum (53.0±2.3) in petrol ether fraction followed by ethanol (52.6±3.1) and methanol (41.5±3.4). The other fractions exhibited low polyphenolic content (30.6-40.8) mg/g of dry extract.

GC-MS analysis confirmed the presence of 13 components by direct similarity searches for *Piper nigrum*. These numbers may be extended with the help of chemometric techniques. *Piper nigrum* seed contained mainly piperine (46.10%), copaene (12.25%) and adamantane (8.07%). The other components were present in low percentages (0.65-5.72).

Phytochemical analysis of fractions revealed the presence of alkaloids and phenolics as major group of compounds. The total phenolic content equivalent to gallic acid (mg/g of dry extract) showed polyphenolic content of 50.1±3.9 mg in ethanol fraction followed by the other fractions which ranged from 34.7 to 37.7 mg/g of dry extract.

The GC-MS analysis of *Piper cubeba* ethanol fraction revealed 15 chemical components which may be extended with the help of chemometric techniques. The major compounds identified were copaene (13.47%), napthalene, 1,2,3,5,6,8a-hexahydro (10.36%) and ledol (6.25). The α-cubebene and other compounds were present in low percentages ranging from 0.18-4.54.

**Conclusion**

The findings of this investigation may be concluded as follows.

I. Multi drug resistance in bacterial population is a common problem. The major mechanisms of resistance detected are β–lactamases and mecA gene product in MRSA strains while extended spectrum β–lactamases (ESβL) production in enteric bacteria. Other mechanisms of resistance like efflux pump are also expected.
II. Screening of selected Indian medicinal plants showed that almost 50% (12) of these plants have broad spectrum antibacterial activity.

III. A total of 9 selected plants showed promising broad spectrum antibacterial activity in order of \( P.\) granatum > \( C.\) copticum > \( C.\) zeylanicum > \( M.\) koenigii > \( S.\) aromaticum > \( Z.\) officinale > \( P.\) guajava > \( P.\) nigrum > \( P.\) cubeba against MRSA and ESβL producing multidrug resistant strains. These fractions need further characterization to identify anti-resistance compounds.

IV. Seven plants were fractionated using different solvents and different fractions were found active against most of the test bacteria. Overall \textit{in vitro} efficacy of various fractions was found in the order of methanol fraction of \( P.\) granatum > \( C.\) copticum (methanol) > \( M.\) koenigii (benzene) > \( Z.\) officinale (ethyl acetate) > \( P.\) guajava (methanol) > \( P.\) cubeba (ethanol) and \( P.\) nigrum (methanol) fractions.

V. Antioxidant activity of 25 medicinal plants by four different \textit{in vitro} assays demonstrated that most of these plants are potential antioxidants. The broad spectrum antioxidant activity was shown by \( A.\) cepa, \( C.\) copticum, \( C.\) cyminum, \( C.\) longa, \( L.\) nobilis, \( M.\) koenigii, \( M.\) fragrans (aril), \( M.\) fragrans (seed), \( P.\) guajava, \( P.\) granatum, \( S.\) aromaticum and \( Z.\) officinale.

VI. Out of seven fractionated plants, five plants viz. \( C.\) copticum, \( M.\) koenigii, \( P.\) guajava, \( P.\) granatum and \( Z.\) officinale were effective and represented strong antioxidant potential in one or more fractions.

VII. Lack of \textit{in vitro} toxicity and mutagenicity in Ames test are an apparent indication for non toxic nature of selected plant fractions.

VIII. The most antioxidant active fractions were emerged as potential source of antimitogenic agents.

IX. Phytochemical analysis by colour test and infrared spectroscopy (IR) analysis revealed the presence of phenolics as major groups of compounds together with other compounds in one or more plant fractions in different combinations.

X. HPLC, GC-MS and LC-MS analysis of selected bioactive plant fractions revealed the presence of punicalagins and ellagic acid in \( P.\) granatum, thymol in
Summary

*C. copticum* caryophyllene in *M. koenigii*, 3,6-dimethyl-2,3,3a,4,5,7a-hexahydro and gingerol in *Z. officinale*, 4-methylthiazole in *P. guajava* as major bioactive components. These phytoconstituents showed activity either alone or in combination of other minor constituents.

XI. The antioxidant and antimutagenic activity of plant fractions further highlights the promising potential of traditionally used Indian medicinal plants and requires further attention to exploit in mutation related diseases including cancer as well as neurodegenerative diseases.

It is interesting to note that in majority of the plants; most active fraction exhibited more than one activities (antibacterial, antioxidant and antimutagenic). Thus, compounds with multiple therapeutic potential are to be identified for treating complex diseases. Moreover, the role of synergistically interacting compounds is also needs to be explored. Alternatively, extracts and partially purified fractions may be tested *in vivo* for their efficacy, safety after standardization and development of improved quality of herbal formulation based on single or multiple therapeutic properties of plant extracts.